Molecular Mimicry of Anti-migraine Drugs with the Neurotransmitters, Dopamine (DA) & Serotonin (5-HT) and its Role in the Treatment of Migraine

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Key words

Molecular mimicry, serotonin receptors, dopamine receptors, serotonin, dopamine, tyramine, monoaminergic neurons, migraine, migraine treatment, G protein-coupled receptors, neurotransmission, synaptic regulatory receptors and transporters, nociception, nociceptive afferents, neurovascular disorders, brain nuclei, trigeminal nervous system, trigeminal ganglion, vasoactive biochemicals and neuropeptides*, insilico* molecular modelling.

Dedicated to the fond memories of my past professional life which shaped me to be who I am today, to my family & loved ones who have supported me through my struggles, to my aspirations to be a successful professional, and to my dreams: to be a committed family person, a contributor to the society by climbing the ladder of science and thus becoming a high achiever in life with the help of all my supporters!

Abbreviations

AA	Amino acid
AD	Aldehyde dehydrogenase
AP	Action potential
β_2AR	β_2 adrenergic receptors
β-AR	β-Arrestin
BBB	Blood brain barrier
CGRP	Calcitonin gene-related peptide
CM	Chronic migraine
CNS	Central nervous system
CTTH	Chronic tension-type headache
D_1	D_1 subtype of the dopamine receptors
D_2	D_2 subtype of the dopamine receptors
DA	Dopamine
DHE	Dihydroergotamine
ERK	Extracellular signal–regulated kinases
ERM	Ergotamine
G protein	Guanine nucleotide binding protein
G score	Glide score
GABA	gamma-Aminobutyric acid
GIRKs	G protein-coupled inwardly-rectifying potassium channels
GPCR	G protein-coupled receptors
GPCRs	G protein-coupled receptors
GPs	General practitioners
GRKs	G protein-coupled receptor kinases
5-HIAA	5-Hydroxy indole acetic acid
5-HT	5-Hydroxytryptamine (serotonin)
5-HTP	5-Hydroxytryptophan
5-HTRs	5-Hydroxytryptamine receptors (serotonin receptors)
IHS	International Headache Society
LBC	Ligand binding cleft

LGICs	Ligand-gated Ion Channels
MAO	Monoamine oxidase
MAPK	Mitogen activated protein kinases
MOA	Mode of Action
MRI	Magnetic resonance imaging
NE	Noradrenaline (Norepinephrine)
NO	Nitric Oxide
NSAIDs	Non-Steroid Anti-Inflammatory Drugs
PACAP	Pituitary adenylate-cyclase activating peptide
PAG	Periaqueductal gray
PDB	Protein Data Bank
RVMM	Rostral ventromedial medulla
SERT	Serotonin reuptake transporters
SSS	Superior sagittal sinus
STT	Spinothalamic tract
TAAR1	Trace amine-associated receptor 1
TH	Tryptophan hydroxylase
TRP	Tryptophan
TRY	Tryptamine
VIP	Vasoactive intestinal peptide

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Abstract

Migraine is now perceived to be a complex neurovascular disorder prevalent in a specific age group of young to middle age population. Although the underlying cause of this condition is still not clearly understood; current scientific thinking suggests the involvement of both the serotonergic and dopaminergic pathways. An attempt is made through this research to throw some light on the pathogenesis of this disorder by exploring the role of neurotransmitters, serotonin (5-HT) & dopamine (DA), and the possible activation of their pathways by molecules that mimic them structurally at their receptors. We have explored *in-silico*, the possibility of anti-migraine drugs mimicking 5-HT and how they interact with 5-HT receptors; by obtaining their docking scores and glide scores, which are indicative of these drugs mimicking 5-HT at 5-HTRs and allowing a successful interaction at specific receptor conformation. There is also a possibility of similar molecular mimicry from other sources such as specific foods resulting in dyshomeostasis of important biochemicals such as 5-HT or DA in our body system. We hypothesise that 5-HT or DA dyshomeostasis together with other genetically predisposed contributors and vasoactive biochemicals can sensitise or activate the brain centres to trigger migraine response. Anti-migraine drugs which can activate these monoaminergic receptors are doing so because they are able to mimic their natural ligands, and by doing so they might possibly correct this 5-HT or DA dyshomeostasis partially, which is subject to its availability in the affected CNS region. Structural analogy of the anti-migraine drugs to the neurotransmitters, makes it a good reason to explore their role in correcting such underlying dyshomeostasis, which might explain their drug efficacy and thus hinting at a scope to develop a targeted treatment for migraine in the future. We hope this research work on the potential drug analogy to neurotransmitters, helps contribute to the improvement in effective management of this complex neurovascular disorder.

Chapter 1 Introduction

1 Introduction

1.1 Background

Migraine is a primary episodic headache disorder characterised by neurological, gastrointestinal, and autonomic changes (1). The word migraine is derived from the Greek word hemicrania, first used by Galen in 200 AD; 'Hemi' means half in Greek, and 'Crania' means skull, as migraine is characterised by pain in one half of the head. 'Hemicrania' over the years transformed into 'migraine' as we know today.

The unique symptoms of migraine distinguish the disorder from other manifestations of headache. For example, migraine is characterised by neurobiological events such as blurred vision, a wave of cortical neuronal activity depression spreading during a migraine with aura (usually visual, however can be other sensory or verbal disturbances) and hypersensitivity to sensory stimuli (e.g., sound, light, smell). Indeed, migraine sometimes is not associated with headache per se, but manifests as aura alone.

There are a multitude of theories among scientists to explain the aetiology of migraine. However, it is widely accepted that certain triggers contribute to the onset of migraine, such as decreased serum oestrogen levels (e.g., post ovulation (2)), stress factors (e.g., anxiety (2, 3)), or certain food cravings (e.g., cheese, coffee, chocolate, peanut butter, red wine (4, 5)). Components of these foods (e.g. caffeine in coffee) are believed to initiate the onset of migraine by triggering symptoms, for example, cortical spreading depression (CSD) (2, 4, 5). CSD involves a sudden change in the ion homeostasis of cortical neurons and is characterized by severe spreading of neuronal excitation followed by an extended period of neuronal depression (2) (also see section 1.4.10). It is possible that this neuronal excitation phase leads to food cravings to redress a biochemical imbalance.

Migraine is now considered to be a complex neurovascular primary headache disorder (as defined by the International Classification of Headache Disorders (6)), common among young to middle age population. Interestingly, migraine is more commonly reported

among females than males, during their lifetime: prevalence increases in females rapidly after puberty while the same rapid increase is not seen in males (7, 8). This increased prevalence of migraine continues after puberty in males and females, up to 40 years of age then declines; however, before puberty the prevalence is similar in both genders (7, 8). Stable serum oestrogen levels have a protective effect against the recurrence of migraine in women. For example, the hyper-oestrogenic state during pregnancy or during lactation is associated with a decreased incidence or improved symptoms of migraine (2). Migraine can be highly debilitating and seriously impact the wellbeing, quality of life, and the economic status due to increased healthcare costs, reduced income due to missed work days or other missed earning opportunities.

The above impacts on life are difficult to ameliorate because there is no simple cure or 100% effective treatment yet available for migraine. For a long time, migraine has been treated symptomatically. A complete cure is likely a long way off, as the root cause is still not completely understood. With advances in our understanding of the biochemistry underpinning migraine, we might expect developments in the treatment options and improved outcomes for migraine sufferers.

Most of us have suffered headache at some point in our lives. Some migraine sufferers simply get used to the fact that they cannot stop migraine from recurring and do not visit their doctor thus receiving no formal treatment. For this reason, the full extent of migraine occurrence is probably not known for the general population. Despite all this, headache (including migraine) remains the 3rd most common cause of missed work, and has a significant impact on life in general (8) .

1.1.1 History

Since the dawn of human civilisation migraine is known to have existed: some well-known migraine sufferers from ancient history include Julius Caesar, Charles Darwin, Sigmund Freud and Napoleon to name a few (8). Though not many evidences of treating migraine during the ancient era, a surgical intervention called the trepanation procedure has been

practised since 7000 BC as a treatment for migraine. In this procedure, the skull is perforated with an instrument. Trepanation may have been done to release demons and evil spirits, that were believed to have caused headaches such as migraine, during this period (8). There is evidence of Egyptian prescriptions called the Ebers Papyrus, written in 1200 BC, which describes migraine symptoms (8). Hippocrates described migraine with aura in 400 BC (8, 9) and believed vapours arising from the stomach caused the headache. However, it was also believed by some to be a punishment from God (8, 9). By the 13th century, migraine treatments were diverse, from plant-derived analgesics such as opium to simple vinegar. By the 19th century Charles Darwin suggested centrifugation as a means of treating migraine, because he believed brain vasodilation was the cause of migraine and believed that centrifugal force would force blood out of brain and relieve the symptoms (2, 8, 9).

The first book on migraine was written in 1873 by Edward Liveing, where he proposed a neuronal theory of migraine, describing it as disturbances in the autonomic nervous system (8, 9). Towards the end of the 19th century Sir William Gowers recommended Indian hemp (marijuana) as a treatment for migraine. In 1938 John Graham and Harold Wolff demonstrated for the first time that ergotamine, produced by the ergot fungus (*Claviceps purpurea*), was an effective treatment for migraine because it resulted in vasoconstriction – this also supported the vascular theory of migraine (8, 10). By 1943 dihydroergotamine (DHE) was synthesised by Stoll and Hoffman and was first used commercially for migraine treatment (10): synthetic ergotamines are still used extensively in the treatment of migraine even today.

As of today, migraine treatment is partly determined by the symptoms and the mechanisms thought to be responsible for the symptoms of migraine. There were two theories hotly debated: the vascular hypothesis proposed that migraine headaches were due to vasodilation of the extracranial blood vessels, and the neurogenic theory proposed vasoconstriction of the intracranial blood vessels resulting in neurological events (10). By the late 20th century various schools of thought challenged both the vascular and neurogenic theories. In 1981 a study by Olsen measured oligemic (reduced total blood

volume in this case in a localised region) episodes in which a wave of reduced blood flow originating from the occipital lobe spreads across to the half region of the brain during the episodes of migraine. This suggested that reduced blood flow was not due to vasoconstriction, but rather due to vasoactive neurotransmitters causing vasodilation (11). This sea change in understanding the aetiology of migraine led to the evolution of treatment modalities.

Sumatriptan, which is commonly used to treat migraine nowadays, does not have analgesic properties as such; however, when used in migraine, it relieves symptoms of pain (10). A breakthrough in understanding of triptan's mode of action (MOA) in migraine came out of a sumatriptan study published in 1991 (12). In this study, middle cerebral artery (MCA) flow velocities were measured: it was found that during migraine attacks, MCA flow velocity was reduced due to a 20% increase in artery diameter (i.e. dilation, often termed distended arteries). Sumatriptan improved the flow within 30 min and the dilatation was reversed. Hence, it was believed that migraine headache were due to large intracranial arterial dilatation and that sumatriptan acted predominantly on distended arteries during migraine attacks (10, 12).

1.1.2 Current concepts and pathophysiology of migraine

Although the underlying cause of migraine is still not clearly understood due to varying theories and concepts; current scientific thinking suggests the involvement of serotonergic, noradrenergic and dopaminergic pathways in the central nervous system (CNS) (1, 13-18). These neurotransmitters are key to the activation of the trigeminal nervous system, which is thought to be the cause behind neurogenic vascular events such as CSD and hyperactivation of the sensory stimuli.

The migraine attack progresses through different stages in some patients, which include aura as well as changes in cortical function, blood flow, and neurovascular events. The aura phase overlaps with the headache phase, which is associated with further changes in blood flow, activation of the brainstem, thalamus, hypothalamus, and cortex (14, 19).

Neurotransmitter receptors such as serotonin receptors (5-HTRs), vasoactive biochemicals such as Nitric Oxide (NO), vasoactive neuropeptides such as calcitonin gene-related peptide (CGRP), and other biochemicals such as pituitary adenylate cyclase-activating polypeptide, and prostanoids have been implicated in developing migraine based on therapeutic and triggered migraine studies (18). Hence the neurovascular hypothesis is a good approach to explain various events associated with a migraine attack, and to understand the subsequent amelioration of pain obtained with anti-migraine drugs; for example, sumatriptan produces cranial vasoconstriction and inhibition of the trigeminal nervous system demonstrated in experiments in rats and guinea pig (13, 20).

Based on the clinical features of migraine, there are 4 known stages. Each stage has specific symptoms, and patients may experience all or some of the symptoms (8, 13). These stages are:

- **Prodromal phase:** Symptoms appear a few hours or a day before the actual onset of headache. Symptoms involve feelings of sluggishness, depression, craving for certain foods, increased appetite, etc. This stage can also be considered as a trigger phase due to mixed behavioural and feeling changes. Some think that certain foods can act as a trigger due to mixed food cravings experienced during this stage, and some think that environmental factors contribute to mood changes and can act as a trigger as well.
- **Aura phase:** A few migraineurs (i.e. a migraine sufferer) experience aura with or without headache. There are many forms of aura; the most common kind is visual aura such as scintillating scotoma characterised by disturbed one half of the vision with expanding bright light flashes. The other kind of aura is sensory aura characterised by tingling or numbness sensations on the lips or in one hand. Other auras involve the inability to speak, hallucination of abnormal smell, weakness on one side of the body and visual distortions called ‘Alice in Wonderland syndrome’ based on the writer Lewis Carroll’s imagery from his novel of the same name: Carroll could have been describing his own migraine aura experiences in his story book (8).

- Aphasic aura (i.e. difficulty speaking, reading and writing) and motor aura (i.e. difficulty moving) are also experienced by some.
- **Attack phase:** This is the actual headache phase characterised by pulsating, throbbing or severe pain on one side of the head, along with nausea and/or vomiting and hypersensitivity to light and sound (8). Some experience swelling of their fingertips, difficulty with mental alertness, and memory issues.
- **Postdromal phase:** This is the end phase of migraine; it manifests as tiredness (this is often regarded as a relief), depression, and, for some, it is a relief phase because the debilitating headache is over.

1.1.3 Migraine's link to specific foods and the structural analogy of dietary amino acids to neurotransmitters in migraine

The onset of migraine can be linked to specific foods, which are also known to be the sources of certain dietary amino acids (AAs), such as the biogenic amino form of tyrosine known as tyramine (Fig. 1.1) and tryptophan which is a precursor to serotonin(4, 5, 21-25). Interestingly, tyramine has a structural analogy to certain neurotransmitters such as DA and NE (Fig. 1.2), as these neurotransmitters share a structural commonality with tyramine's molecular structure (Fig. 1.2) (26, 27). Tyramine is found in cheese, chocolate and wine (Fig. 1.1), which many migraineurs report having experienced severe food cravings for, especially during the prodromal phase. However, some researchers believe that these foods are not migraine triggers (8). This structural analogy between AAs and neurotransmitters could mean these AAs have the potential to mimic their 'lookalike' neurotransmitters; for example, in the CNS, these compounds may behave like their lookalike neurotransmitters if they are able to cross the blood brain barrier (BBB). There are also non-food migraine triggers relating to specific situations (e.g., anxiety, depression, stress) or specific environmental conditions (e.g., temperature variations) etc. (26, 28).

Tyramine (Fig. 1.1 & 1.2) is a naturally occurring biogenic monoamine found in cheese and chocolate; it is derived from the amino acid tyrosine via decarboxylation by certain microorganisms in the gut or during the fermentation process of foods (29-31). The striking structural similarity of tyramine (Figure 1.2) to NE & DA could explain why migraine patients relate their onset of symptoms to these specific foods. Hence, it is possible that tyramine substitutes for either NE or DA in the synaptic system, perhaps initiating a neurotransmission which could be the basis of the onset of migraine symptoms (Fig. 1.8; see also Discussion Section 3.2.1). The presence of tyramine in the human brain suggests that they are able to cross the BBB (perhaps via the G protein, trace amine-associated receptor 1 (TAAR1) (32)) and, therefore, one can assume that it can interfere with DA and NE-mediated neurotransmission due to their molecular structural analogy (Fig. 1.2 & 1.9). On the other hand, there is a possibility that tyramine might be formed by decarboxylation of tyrosine in the brain itself rather than crossing the BBB (Fig. 1.3) and therefore besides food sources, tyramine can be formed in the brain itself. The distinction between these two possible sources of tyramine's presence in the brain is not understood (See Discussion, Section 3.2.1). TAAR1 is found in the brain, peripheral tissues, and other internal organs including kidneys and are known to have high affinity for tyramine (32). Hence TAAR1 can be considered as a carrier protein of tyramine. Tyramine is metabolized by monoamine oxidase (MAO) enzyme, however its metabolism can be compromised in the presence of agents such as monoamine oxidase inhibitors (MAOIs). When foods high in tyramine are ingested along with MAOIs, a hypertensive crisis can result, as it can displace stored monoamines, such as dopamine from the neuronal vesicles (32). Thus, a large dietary intake of tyramine (or a dietary intake of tyramine while taking MAO inhibitors) can result in a counter response as tyramine pressor response by the body, which is defined as

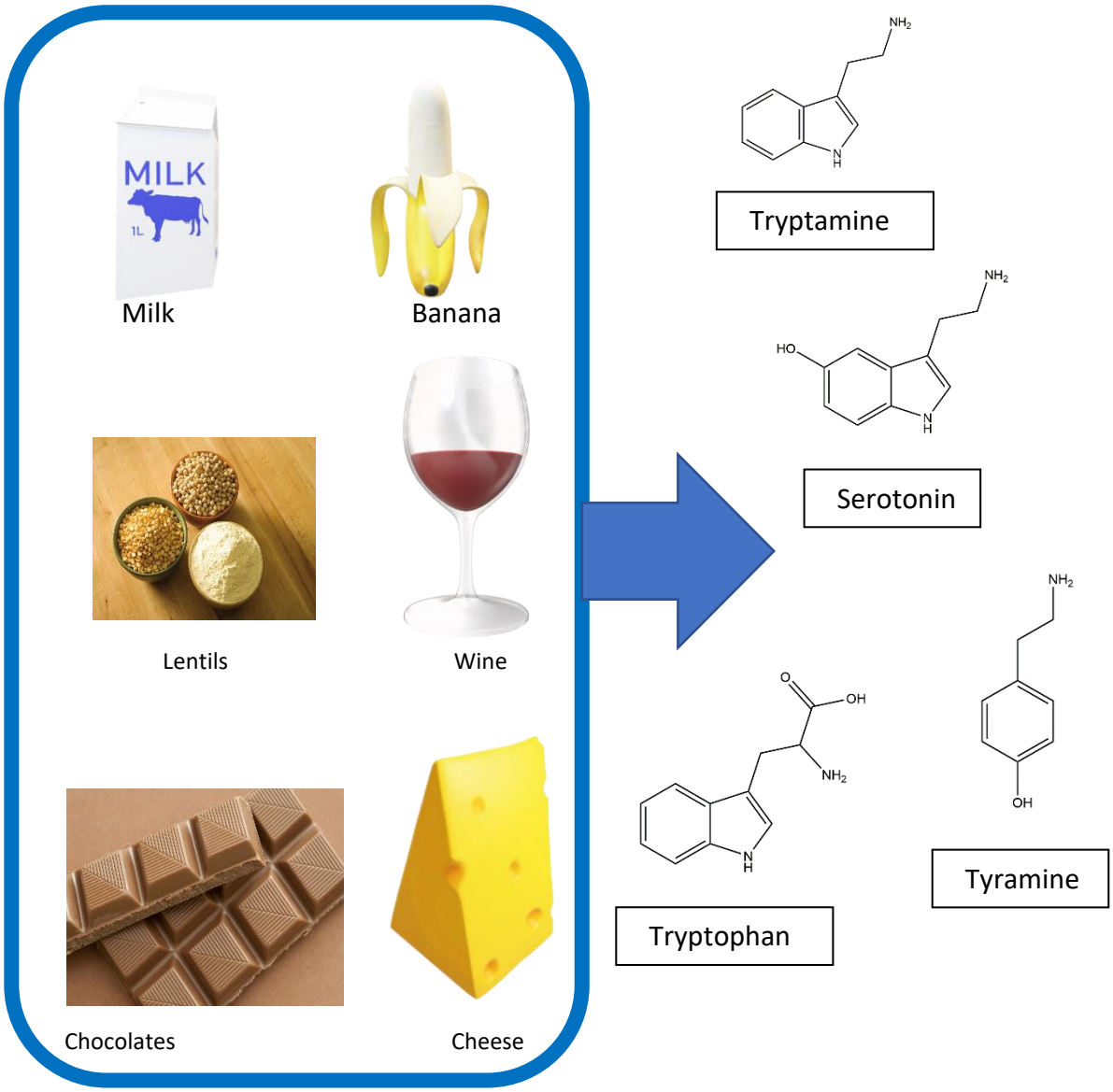


Figure 1.1 Dietary sources of tryptophan, serotonin, and Tyramine.Tryptophan is an essential amino acid, precursor to serotonin (neurotransmitter). Tryptamine is structurally analogous to both serotonin and tryptophan, and similarly tyramine to DA & NE (Figure 1.2). Both tryptamine and tyramine are known to be found in trace amounts in the brain of mammals; however, whether they can cross the BBB is still questionable. Tyramine is known to have high affinity to TAAR1 receptors in brain (32), which might aid in its translocation into the brain.

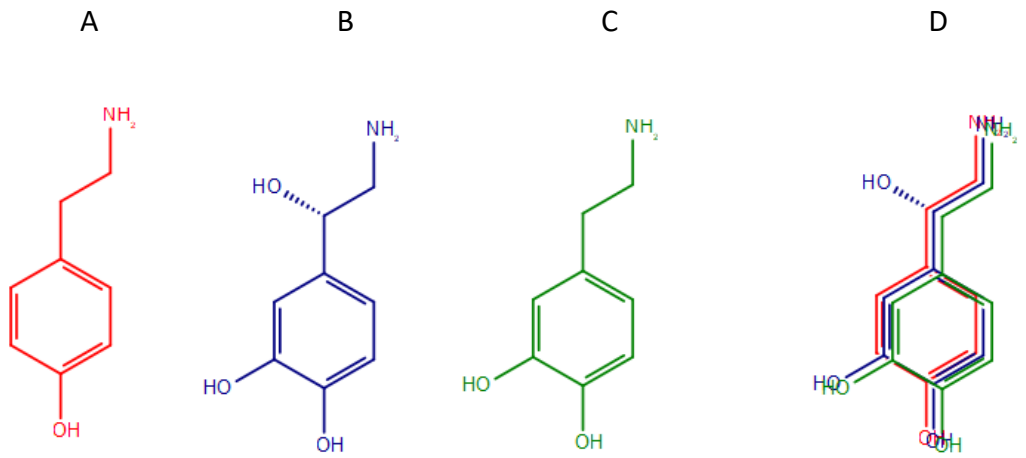


Figure 1.2 Tyramine (A), noradrenalin (B), dopamine (C) and the three molecules superimposed (D) to show their structural analogies.

an increase in systolic blood pressure of 30 mmHg or more (32, 33). The displacement of neurotransmitters other than dopamine, such as norepinephrine (noradrenaline) from neuronal storage vesicles by mimics such as tyramine can also lead to vasoconstriction, increased heart rate and high blood pressure due to a counter pressor response (26, 28, 33). Such a counter pressor response due to the displacement of monoamine neurotransmitters can be understood by the complexity involved in the neurotransmission processes. Nerve terminals involving postsynaptic receptors, pre-synaptic regulatory receptors and transporters as shown in Fig. 1.9 play a crucial role in this transmission mechanism by maintaining a constant or balanced concentration (i.e. homeostasis) of neurotransmitters in the synaptic region as well as in the neuronal vesicles. Maintaining this constant or balanced concentration of neurotransmitters in the synaptic cleft, can be compared to a buffer solution where the pH is maintained even when there are fluctuations in the levels of H^+ ions due to small additions of acids or bases. Similarly, the neuronal vesicles, pre & post synaptic receptors, neurotransmitter degrading enzymes and transporter proteins together give the effect of a buffering mechanism where a balanced level of neurotransmitters at the synaptic cleft is maintained which in turn contributes to the homeostasis of the neurotransmitter level at the synapse and the neuronal vesicles.

Disturbances in this homeostasis (dyshomeostasis) can have significant repercussions, and the mechanisms by which it counter acts to this disturbance is still not clearly understood.

In the absence of neurotransmitter availability in neuronal vesicles, molecular mimics such as tyramine may replace DA neurotransmitters or there could be situation of excess molecules contributing to disturbances in the homeostasis at the synaptic cleft. In such situation, there is a potential to generate a counter response, hence we hypothesise that migraine might be part of such counter response complications to dyshomeostasis.
It is interesting to note that DA, NE and 5-HT, are known to be involved in the pathophysiology and treatment of various neuro disorders. These neurotransmitters are constantly being released into the synapses, to regulate various biological processes in CNS, which is being currently explored extensively by the scientific community. Any change in their systemic level can have reciprocal response or modulatory response between these neurotransmitter systems, for example, an antagonistic effect on the serotonergic system by certain molecules can have an enhanced firing rate of the dopaminergic system and noradrenergic system and vice versa (21). Therefore, we may assume that, molecules selectively targeting one system-specific receptor can indirectly modify the neuronal firing activity of the other two systems.

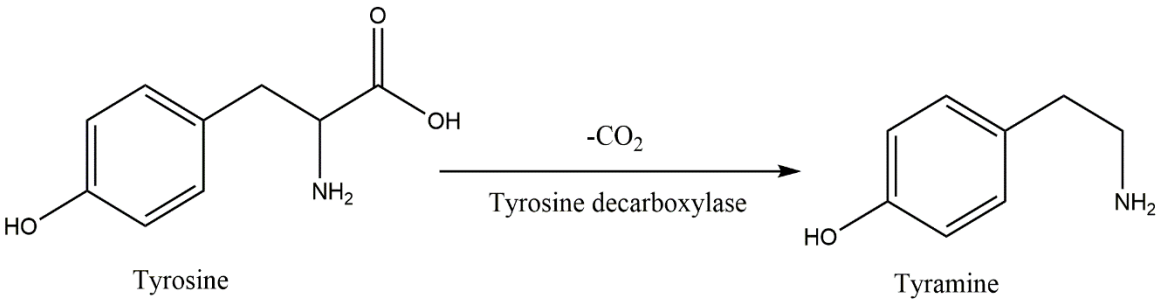


Figure 1.3 Tyrosine AA undergoes decarboxylation in the gut to form tyramine

1.1.4 The Prevalence of Migraine

Based on the Global Burden of Disease Study 2010 & 2015, the International Headache Society (IHS), stated that migraine is ranked as the third most prevalent and disabling disorder worldwide in both males and females under the age of 50 years (6). Many epidemiological studies have documented the impact of migraine from a social, financial and personal perspective and its significant prevalence worldwide. Globally, 46% of the adult population is affected by active headache disorder including migraine in general, of

which 42% reported tension-type headache, 11% migraine, and 3% chronic daily headache (34). Generally, the prevalence of migraine is higher among females compared to males. In a study based on the US population, migraine was reported in 18.2% of females compared to only 6.5% in males (i.e., approx. 3 times more common in females than males) (35). Migraine as mentioned before is often reported from the beginning of adolescence as the hormone level seems to have an influence on the incidence by rapidly increasing it up to 40 years of age then declines probably due to reaching the menopausal stage (35, 36) In females, thus migraine attacks have been linked to hormone levels; for example, in some studies migraine was associated with reduced incidence during pregnancy (2, 37). In the USA, the prevalence of migraine is reported to be approx. 13% of the general population as afflicted with the disorder (35). Migraine can severely impact a person's quality of life, the capacity to contribute at work, or study. These impacts can significantly affect a sufferer's capability, and because migraine is likely to have significantly underdiagnosed and undertreated, the overall social and economic impacts are probably greater than what is expected. The economic impact is also considerable due to missed work days and the high cost of treatment.

Typical symptoms of migraine are headache with or without aura, nausea, vomiting, blurred vision, light headedness sometimes followed by fainting etc with a frequency ranging from once a year to once a week in affected people in general (35).
Data from another study based on the American Migraine Prevalence and Prevention Study, which was conducted on a mailed survey sent out to 120,000 US families, reported the incidence of migraine was maximum between the ages of 20 and 24 years old in women and 15 and 19 years old in men (36). The most interesting findings from the above study were; overall incidence reported was more than double among women (43%) than with men (18%) and the median age for the onset of migraine among women was 25 years and 24 years for men. Four of every 10 women and two of every 10 men will contract migraine in their lifetime, most before the age of 35 years of age (36).

1.2 Migraine and its treatment

Migraine is classified into many different types as per the International Classification of Headache Disorders 3rd edition (ICHD-3), published by International Headache Society (IHS). IHS is a registered charity organisation founded by committed professionals globally and is based in the UK. The next section will highlight the types of migraine for diagnostic purposes and the different treatment options of migraine.

1.2.1 **Types of migraine**

Though there are many types of migraine (Fig.1.4), two categories are most commonly used, migraine without aura and migraine with aura. An example of a subtype of migraine with aura is retinal migraine. As the name suggests it affects the retina of the eye causing visual disturbances including blindness usually accompanied by headache. A subtype of complications of migraine, known as migrainous infarction, occurs in migraine with aura accompanied by ischaemic brain lesions diagnosed with neuroimaging scans. In the case of hemiplegic migraine, aura is accompanied by fully reversible motor weakness symptoms along with visual, sensory and/or speech or language symptoms (6).

Migraine without aura is characterized by headache with specific symptoms, such as with nausea and/or photophobia and phonophobia while migraine with aura is primarily characterized by short term neurological symptoms affecting a specific region of the brain that usually precedes or sometimes accompanies the headache. Some patients experience varying symptoms as they pass through the different phases of migraine (see section 1.1.3) such as prodromal and postdromal phase, which include hyperactivity, hypoactivity, depression, nausea, vomiting, photosensitivity, phonophobia, cravings for specific foods, repetitive yawning, neck stiffness etc. Generally, the diagnosis for migraine headache is made based on the ICHD -3 criteria and excluding secondary headache disorders, where a pre-existing condition such as a tumour is the cause for headache (6, 7, 38). Though there are different classes of migraine for diagnostic purposes, migraine is perceived by people in general as a form of headache disorder with unique symptoms, slightly different from the ordinary headaches for the public as not much is known about the disorder due to lack of

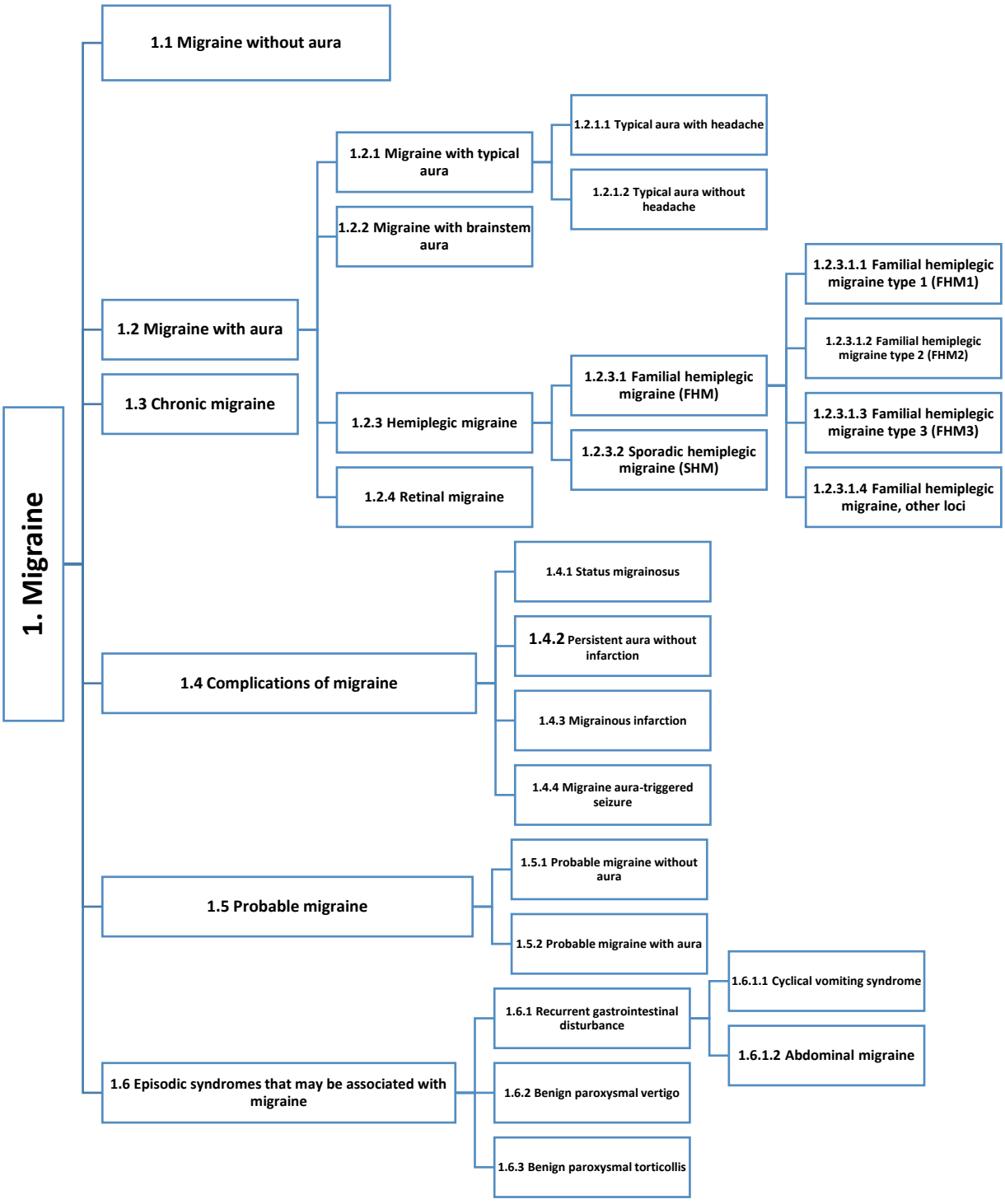


Figure 1.4 Different classes of migraine (table adapted from: https://www.ichd-3.org/1-migraine)
Listing of different classes of Migraine for diagnosis purpose as per the International classification of headache disorders 3rd edition (ICHD-3) (6).

information around the root cause and complexities regarding its pathogenesis. The next section will highlight various treatment options currently available to manage migraine.

1.2.2 Treatment

The treatment options for migraine can be either a preventative approach or an abortive approach (Fig.1.5). There may be several co-existing conditions at the time of diagnosis, such as with secondary headaches (see section.1.2.1.). Educating the patient about the common migraine triggers such as dietary triggers, hormonal changes, irregular sleep patterns, sensitivity to climatic temperature and weather patterns, emotional stress and irregular meal habits is very important in helping them to manage migraine by introducing some life style changes (7, 39). Once the triggers are identified with the help of their medical practitioner, changes in their lifestyles can bring some improvements in the frequency of incidence. Along with life style changes, Non-Steroid Anti-Inflammatory Drugs (NSAIDs), β-blockers, anti-depressants, combination analgesics, dopamine antagonists, corticosteroids, opioids, triptans etc. are medications (Fig.1.5, 1.6 & 1.7) commonly prescribed in migraine. Acute treatment of migraine is most suitable during the prodromal phase i.e., the early headache phase. Use of migraine specific medications were shown to be better, compared to non-specific therapies as demonstrated in figure 1.5. Oral NSAIDs and acetaminophen in combination with caffeine are known to relieve symptoms. Gastric side effects may be the main limiting factor with most of the combination therapies. Careful patient monitoring is required to avoid drug dependency leading to drug overuse symptoms such as, drug overuse headaches, and potential for withdrawal symptoms. 10–20% of the refractory headache patients (who did not respond to common analgesics) subsequently selected for chronic opioid therapy (such as codeine), responded with headache reduction and functional improvement over the long term (1, 7).

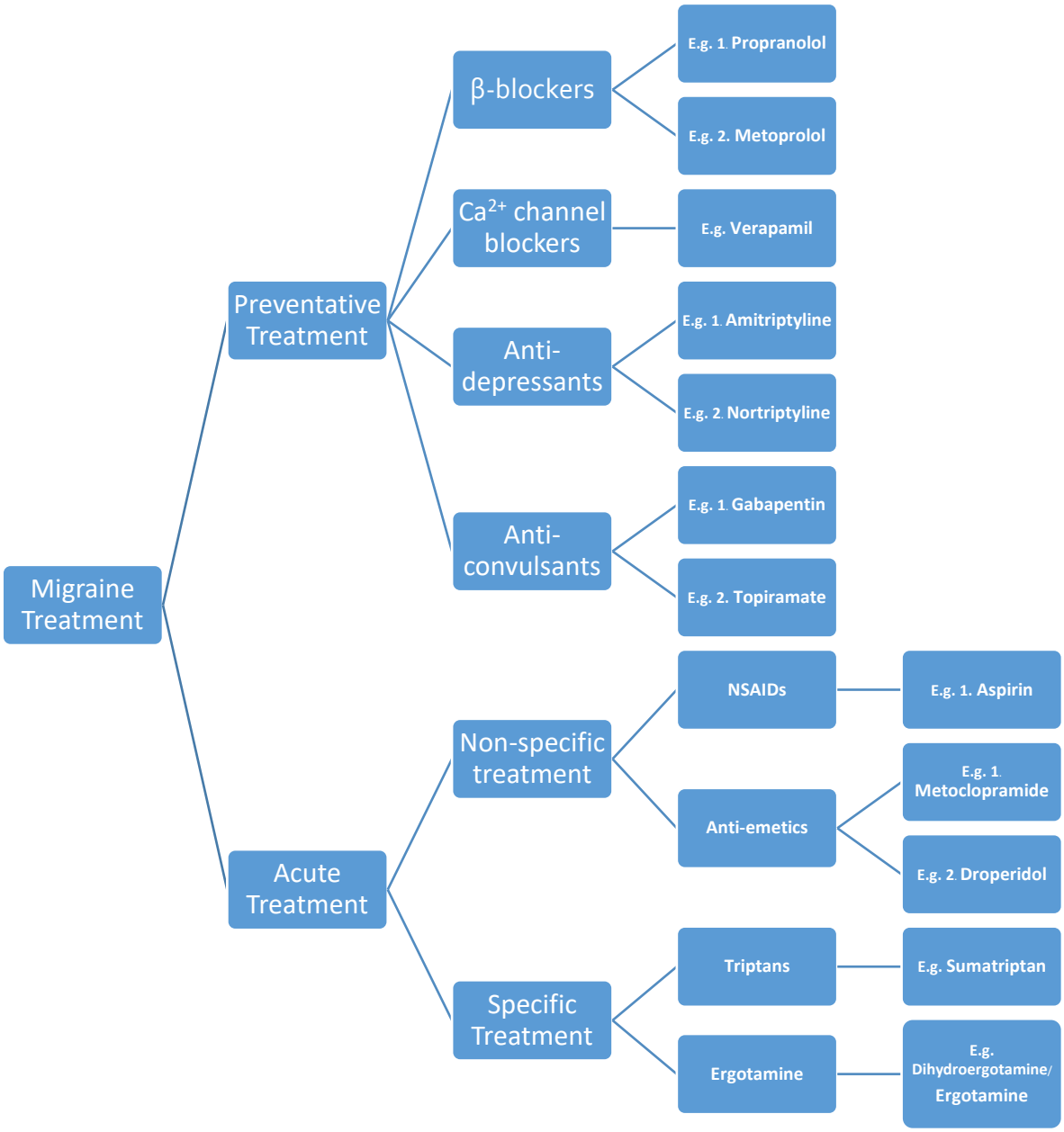


Figure 1.5 Migraine treatment goals and anti-migraine treatment options currently in practise.
A representation of different classes of anti-migraine drugs and their treatment modalities.

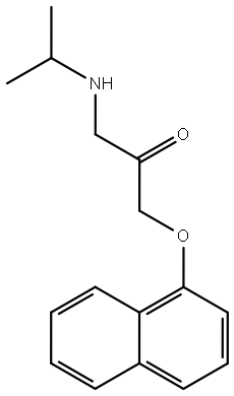
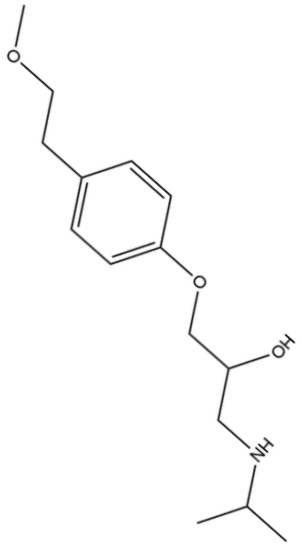
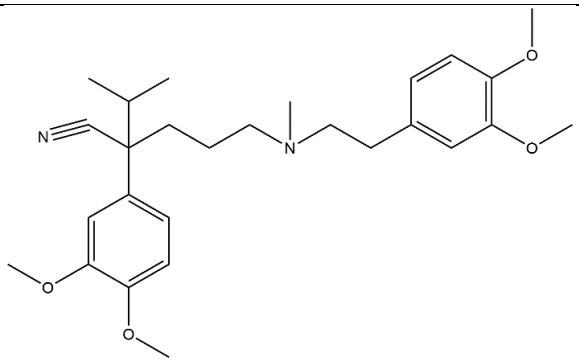
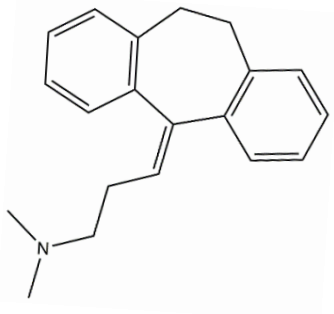
Name of the drug	Molecular structure
Propranolol	
Metoprolol	
Verapamil	
Amitriptyline	

Figure 1.6 List of anti-migraine drugs and their chemical structures.

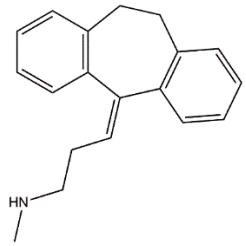
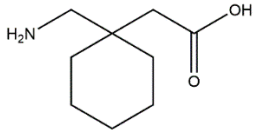
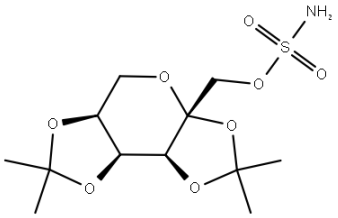
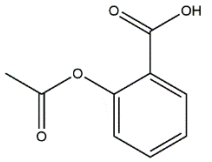
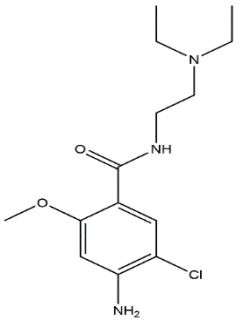
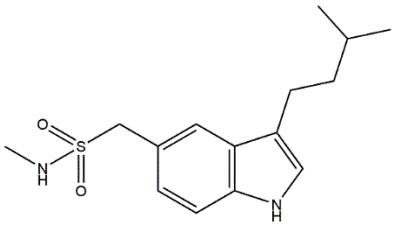
Name of the drug	Molecular structure
Nortriptyline	
Gabapentin	
Topiramate	
Aspirin	
Metoclopramide	
Sumatriptan	

Figure 1.7 List of anti-migraine drugs continued.

Both ergotamine and DHE are known to be migraine specific medications, the same is true with triptans. The first triptan injectable medication: sumatriptan was developed in 1992 as a migraine specific medication. These drugs are known to relieve symptoms due to their vasoconstrictive and neurogenic anti- inflammatory properties (39). Acute anti-migraine effects of triptans are assumed to be attributed due to their agonistic serotonergic properties (40). Over the past 20 years more triptans have been approved by the US FDA are: eletriptan, almotriptan, frovatriptan, zolmitriptan, naratriptan, and rizatriptan. There are some concerns regarding their cardiovascular side effects and drug interactions when co prescribed with other medications. Most of the triptans are available as oral tablets, while a few are also available as nasal sprays like, sumatriptan and zolmitriptan. Certain neuroleptics like metoclopramide are found to be effective when co-prescribed to relieve symptoms of nausea (7). Figure 1.5 represents a sample migraine treatment model, currently practised globally, drafted based on the above information.

1.2.3 Current treatment success with anti-migraine treatment

A published meta-analysis study of anti-migraine treatment results from 24,089 patients in 53 controlled clinical trials of triptans demonstrated that triptans were an effective treatment of choice, and that some triptans were better choice over others in terms of sustained freedom from pain (40). These response rates were higher with higher doses of drugs such as rizatriptan 10 mg, eletriptan 80 mg, and almotriptan 12.5 mg than with their respective lower doses and all the responses were compared with sumatriptan 100 mg as shown in Figure 1.8 (40).

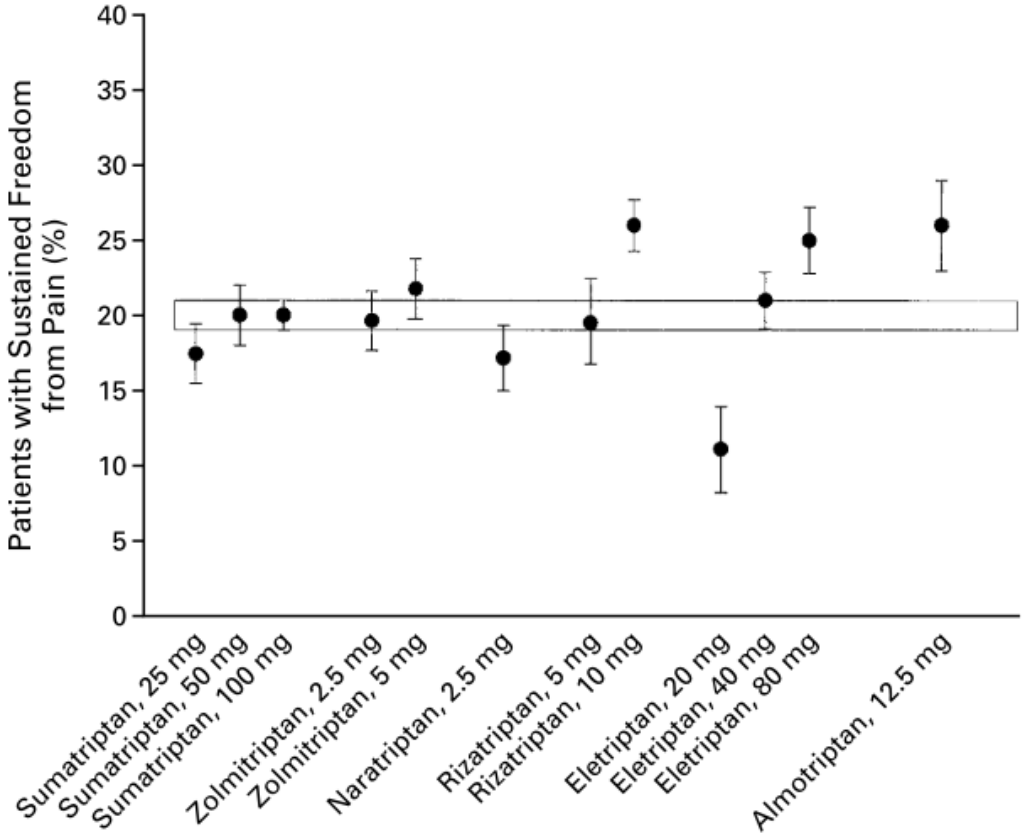


Figure 1.8 A meta-analysis data of treatment responses to anti-migraine drugs (Images reproduced from the New England journal of medicine 2002, by Goadsbey et al. with copyright permission to reproduce in academic works). The boxed region represents response of reference drug - sumatriptan 100 mg at 95% confidence interval (40).

Since lower doses of eletriptan had poor results it could be assumed that at higher doses, both drug availability and drug distribution might have improved the drug response. For improved drug response, the drug needs to cross the BBB and activate 5-HTRs in the brain. As we notice there is a significant response at 80 mg v/s 20 mg of eletriptan. Could this be contributed due to the drug being able to activate a few more of the 5-HTR's in the brain? As it has been reported with other anti-migraine drugs like Aspirin and Sumatriptan, traces of the drugs were detectable in the CNS fluid following oral administration (40, 41).

A meta-analysis report derived from 88 different multinational randomised double blinded clinical trials conducted and published between 1993 and 2016, had clinical outcome analysis of NSAIDs and triptans as the most preferred treatment option in migraine, with a total of 34, 850 participants (42). The authors of this meta-analysis concluded that,

eletriptan may be the most suitable therapy for migraine and ibuprofen might be a good alternative as its tolerability was better with patients (42). Sumatriptan 100 mg and 50 mg, provided good efficacy and tolerability and by far the longest clinical experience recorded since its introduction. The 6 mg subcutaneous formulation of sumatriptan is the most effective for acute migraine treatment, however it is also associated with more intense adverse events (AEs) and the need for self-injection to avoid daily clinic visits. Naratriptan 2.5 mg offers very good tolerability and can be useful in patients with mild or moderate migraine. Zolmitriptan was a good treatment alternative in many patients with migraine symptoms; however, this may not offer any specific advantage over other triptans. Frovatriptan cannot be fully judged in view of the lack of data but does not seem to offer any advantage over sumatriptan either (40, 43).

The results of the meta-analysis study from 53 clinical trials (12 unpublished) involving patients with oral triptan medication, have shown that rizatriptan (10mg), almotriptan, and eletriptan (80mg) have a better response compared to sumatriptan in terms of headache response at 2 hrs and total pain-free responses (44). All triptans in general have better responses than placebo, however there have been concerns regarding their potential for cardiovascular side effects like coronary vasoconstriction, hence precautions were required for patients with cardiovascular complications. Sumatriptan is the first and most widely prescribed triptan, in most of the European countries and North America (44). Data from the above meta-analysis suggests that mean absolute response with sumatriptan 100 mg for headache response were approximately 60% and zolmitriptan, rizatriptan eletriptan 80 mg and almotriptan were better treatment choices. However, mean absolute pain free responses for sumatriptan 100 mg were only less than 30% and zolmitriptan 5 mg, rizatriptan 10 mg, almotriptan and eletriptan 80 mg showed higher response than sumatriptan 100 mg. What is noticeable among the treatment responses is the increase of responses with higher doses of some drugs especially eletriptan 80 mg over, 20 mg is of high interest as we could assume that at higher doses, more traces of the drugs are able to cross the BBB and detectable in central nervous system (CNS) fluids. This is also true with other anti-migraine drugs as aspirin and sumatriptan have shown traces of the drug available in CNS fluid after administering the

drug orally (41). The mode of action (MOA) of anti-migraine drugs, specifically triptans, is believed to involve 5-HTR's (44), which suggest exploring the role of neurotransmitters such as 5-HT in migraine would add to a better understanding of this disorder.

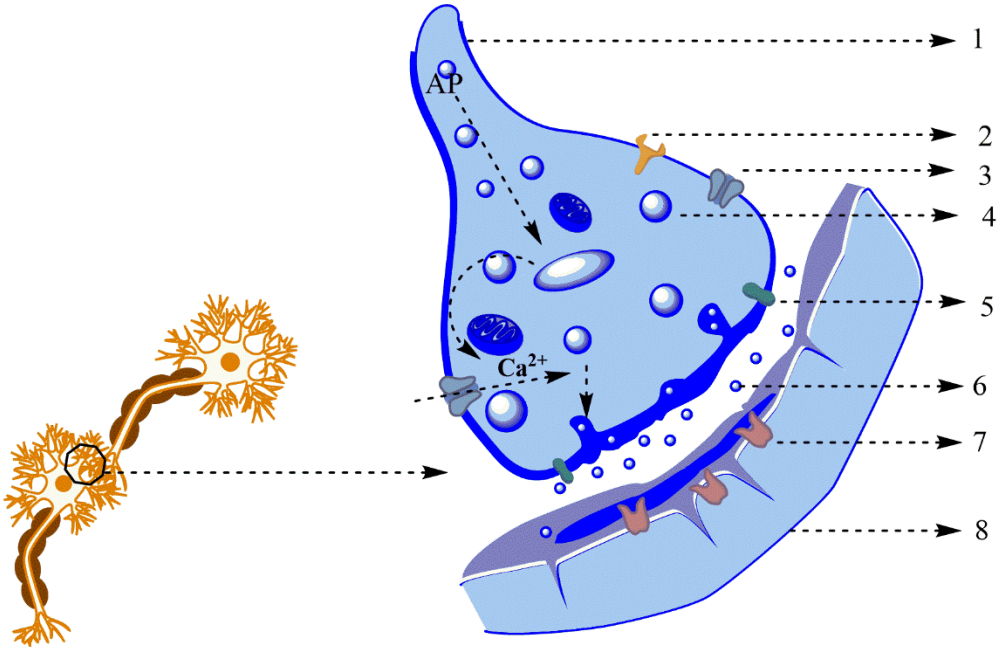
1.3 The role of neurotransmitters in migraine

The role of neurotransmitters (DA & 5-HT) in migraine and its significance in triggering migraine, would be an interesting topic to research, more so due to their structural analogy to dietary amino acids, and their role in neurobiological symptoms such as hypersensitivity to sensory stimuli. Otto Loewi discovered the first neurotransmitter in 1926, during a lab demonstration, wherein he noticed acetylcholine acted as a chemical messenger via the vagus nerve to the heart in lowering the cardiac rhythm by activating certain receptors. Since then, several biochemicals and their respective receptors have been researched in synaptic transmission (45).

1.3.1 Neurotransmitters in synapses

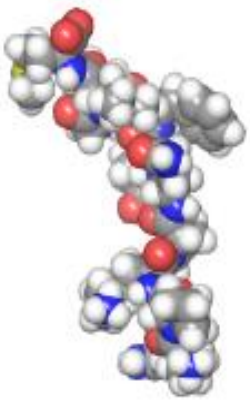
Neurotransmitters play a crucial role in facilitating the communication channels within the CNS via its (neurotransmitter's) release into synapses. Communications within the brain and between brain & the rest of the body are facilitated via this neurotransmission involving synapses (Fig.1.9); which are the contact junctions between adjacent neurons. Neurotransmitters could be made up of mono amines, AAs or peptides. Examples of monoamine neurotransmitters are 5-HT, DA, NE, epinephrine, histamine, etc., and some AA that act as neurotransmitters are glycine, aspartate, glutamate, etc. Somatostatin and substance P are peptide neurotransmitters (Fig 1.10). Neurotransmitters are usually biosynthesised from their precursor amino acids, e.g., 5-HT biosynthesised from tryptophan (Fig. 1.11). There are a vast number of 'biochemicals' endogenously synthesised in neurons and stored within neuronal vesicles, which then act as neurotransmitters upon release into the gaps between neurons at the synapse called the synaptic cleft (or synaptic gaps); except gaseous neurotransmitters such as CO and NO, which are not stored in neuronal vesicles. Upon stimulation by an incoming AP or nerve impulse to the neuron, Ca^{2+} channels on the cell membrane open to allow Ca^{2+} entry into the neuron, which in turn activates neuronal vesicles to move towards the synaptic cleft to allow neurotransmitter release. Upon release into the synaptic cleft, the neurotransmitters

bind to specific receptors on target neurons or may get metabolised by enzymes present in the synaptic cleft or recycled back to pre-synaptic neurons via transporter proteins

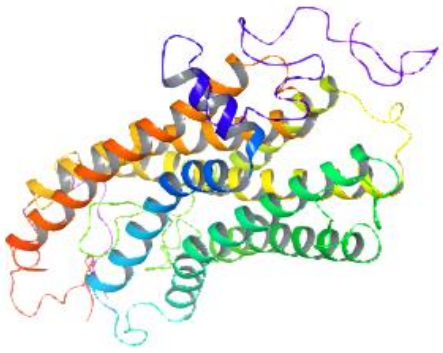


1. Pre synaptic neuron
2. Tryptophan transporter
3. Ca^{2+} channel
4. Neuronal vesicles
5. 5-HT Transporter
6. 5-HT
7. 5-HT receptor
8. Post synaptic neuron

Figure 1.9 A schematic representation of an enlarged inter-neuronal junction called synapse, where transmission of neurotransmitters mediate communication network within central nervous system. An incoming action potential (AP) initiates the Ca^{2+} entry to neuron, which then stimulates neuronal vesicle to move towards the synaptic cleft and diffuse with the cell membrane to release the neurotransmitters into the synaptic cleft.



Substance P



Substance P receptor

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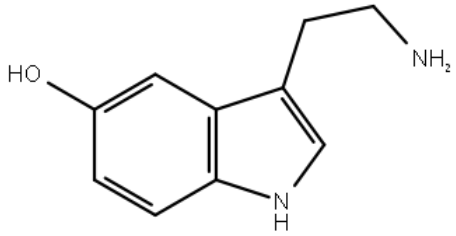
5-HTR



5-HT

Figure 1.10 Examples of neurotransmitters (substance P & 5-HT) and their respective receptors.

embedded on the cell membrane of presynaptic neurons (Fig. 1.10). Post synaptic neurons upon receiving the neurotransmitter-receptor binding, activate these receptors to initiate further changes in the postsynaptic neurons and propagates the action potential (AP) down the line. This activation of receptors and conduction of AP goes on until the CNS communication reaches the destination, and the subsequent desirable changes happen further down the process. During this process of neurotransmission, the signals can be modified (the signal intensity can be enhanced or reduced) or stopped by other intervening receptor proteins, such as gamma-aminobutyric acid (GABA) receptors and glutamate receptors from neurons at synapses (Fig. 1.20). 5-HT is one such neurotransmitter which is released in many serotonergic neuronal synapses as part of the communication channel between the brain and other body parts (e.g., smooth muscles on blood vessels) and are biosynthesised and stored in neuronal vesicles.

1.3.2 5-HT biosynthesis and degradation

Serotonin is biosynthesized, from the amino acid tryptophan (Fig.1.11), which undergoes hydroxylation via tryptophan hydroxylase (TH) enzyme to give rise to 5-hydroxy tryptophan (5-HTP) which is then decarboxylated to serotonin in the presence of enzyme, aromatic amino acid decarboxylase, then later metabolized to 5-hydroxy indole acetic acid (5-HIAA), via enzymes MAO and AD (aldehyde dehydrogenase) (46). 5-HT can also alternately metabolise to a different product as melatonin in brain which aids in sleep (47). Serotonergic neurons store the biosynthesised 5-HT in their neuronal vesicles, upon stimulation by AP signals, 5-HT is released into the synaptic cleft, as described in section 1.3.1 (Fig. 1.9). Excess 5-HT from the synaptic cleft is recycled back to neuronal vesicles via transporter proteins on the presynaptic neuron and the non-recycled 5-HT, is then degraded (Fig. 1.11) and removed from the system. Similarly, dopaminergic neurons have DA stored in place of 5-HT in their neuronal vesicles as described above is released into the synapses upon activation by AP. Neurotransmission processes involving 5-HT & DA are important because they play a crucial role in maintaining a good overall mental health (reduces anxiety), maintaining alertness, relaxation, and calming effects (48). DA is known to have effects on cognitive function and mental sharpness while 5-HT on relaxation, and calming effects (49, 50). 5-HT/ DA are synthesized in presynaptic neuron and stored in vesicles as mentioned earlier, on stimulation they are released into synapses, where they must bind to their post synaptic neuroreceptors to initiate various signalling mechanism to relay their effect. Excess 5-HT/ DA are either degraded via enzymes or recycled back to pre-synaptic nerve via their transporters. The serotonin receptors are activated by the neurotransmitter serotonin (5-HT), which acts as their natural ligand.

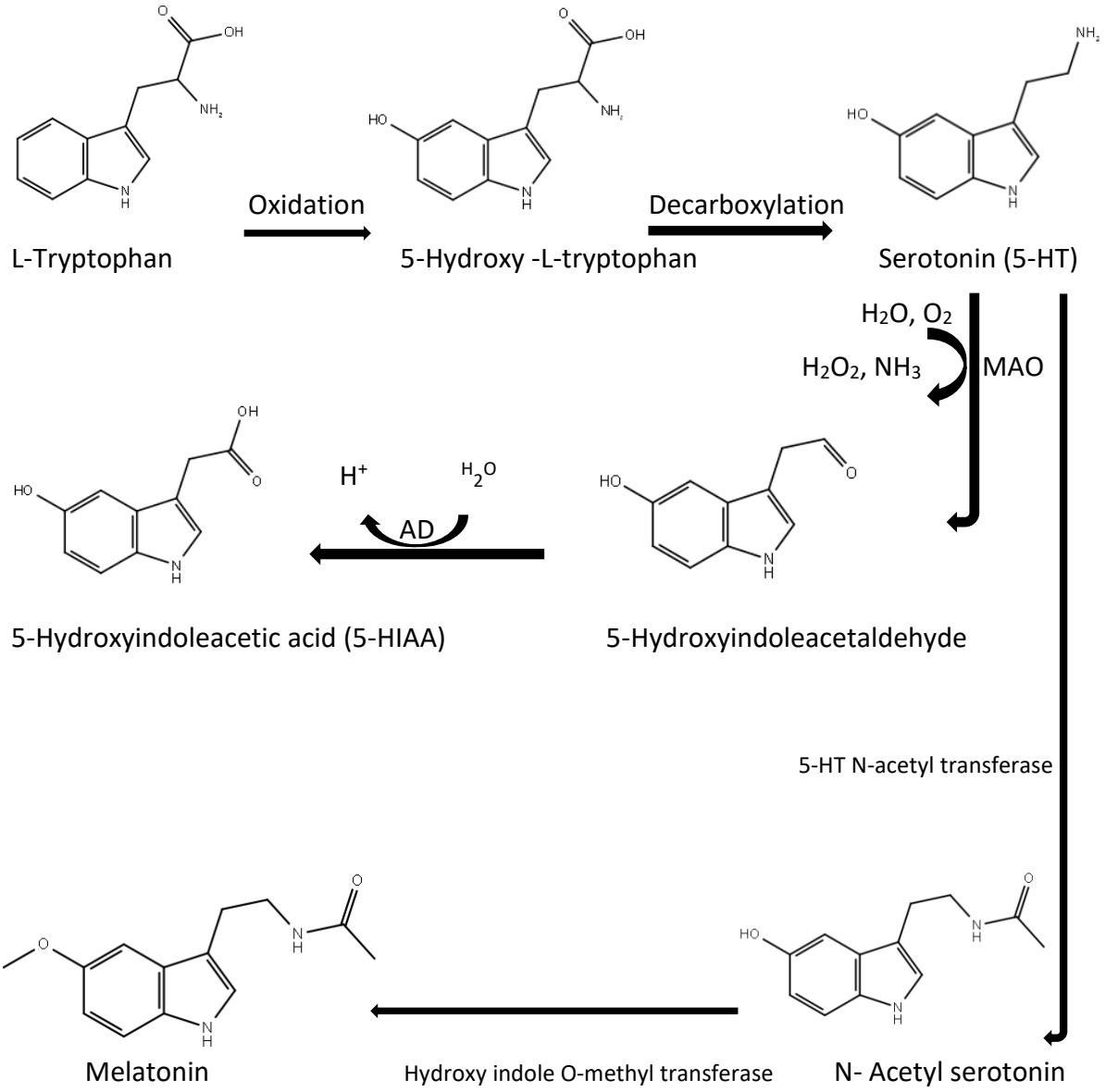


Figure 1.11 Biosynthesis of Serotonin and degradation to 5-HIAA; alternately 5-HT can be metabolised to melatonin as well which aids in sleep.
MAO (Monoamine oxidase), AD (Aldehyde dehydrogenase).

5-HT does not cross the blood brain barrier and hence dietary supplements are not likely to have any effect on altering their availability in brain, however dietary tryptophan is linked to elevated availability in brain (51), as they are a precursor to 5-HT. The same is also true with DA, as it cannot cross the BBB. AAs such as phenylalanine and tyrosine act as precursors to DA biosynthesis and DA itself is known to be precursor to NE. Thus 5-HT, DA & NE are important neurotransmitters, which aid communication between the brain and

the rest of the body as well as a regulatory role between them by influencing each other's level in the system.

In several migraine studies plasma levels of 5-HIAA and 5-HT are studied (48), to understand their role implicated at various stages of migraine. When 5-HT is released into the synapse, they bind to specific receptors on post synaptic neurons, to initiate signalling processes depending on the receptor subtypes they activate. The same is true with DA and NE.

1.3.3 5-HT & DA receptors and their subtypes

5-HT receptors known as 5-hydroxytryptamine receptors (5-HTRs) are localised mainly in the gastrointestinal tract (in enterochromaffin cells) (52) and in brain nuclei (raphe nucleus) more frequently (53). 5-HTRs are a group of G protein-coupled receptors (GPCRs) and ligand-gated ion channels (LGICs) found in the central and peripheral nervous systems (54, 55). These receptors initiate signalling mechanism upon activation by the specific neurotransmitters as their natural ligands and depending on the receptor subtype, they could initiate an appropriate signalling mechanism, and pass on the AP, which is most likely due to depolarisation of the post synaptic nerve, where the permeability to certain ions changes due to the signalling process initiated by ligands via the receptors, which is still not fully understood and is an extensively researched topic (56). 5-HTR signalling down the line, can modulate the release of many neurotransmitters, including DA. Thus 5-HTRs are activated by their natural ligand 5-HT, which is also a neurotransmitter. and the same is true with DA receptors and DA. DA- or 5-HT-mediated synapses exist between nerves and blood vessel walls, resulting in agonistic or antagonistic activation of their respective receptors depending on the receptor subtypes it activates, as some receptor subtypes are inhibitory in nature and some are excitatory in nature (54, 56-59). Both DA and 5-HT receptor subtypes are extensively researched by scientists today and more research is still needed to fully understand their signalling mechanisms. The intensity of neurotransmitter signals from the synapses could be modified further, as mentioned earlier, by certain signalling proteins embedded in the nerve cell membrane such as glutamatergic or GABAergic post synaptic nerves in synapses as mentioned earlier in section 1.3.1.

DA/5-HT receptors are known to have several subtypes, and are located throughout the brain, spinal cord, blood vessels and peripheral nerves. DA/5-HT receptors in the brain, are implicated in intracranial vasoconstriction. On the other hand, intracranial vasodilatation is thought to be involved in the activation of the trigemino-vascular system which stimulates the release of vasoactive sensory neuropeptides, especially calcitonin gene-related peptide (CGRP) from trigeminal ganglia cells, which increases the pain response, and can be an important pathogenesis process during migraine (48). An agonistic activation, of 5-HT receptors in intracranial blood vessels, is indirectly involved in stopping the stimulation of this above pathogenesis process and production of CGRP (48).

Sokolov et al. (2011) (48) suggested there are at least 15 units of known serotonin receptors, of which only a few could be implicated in the pathogenesis of primary headaches and, particularly in the case of migraine headaches; such as 4 units of 5-HT1 type, e.g. $5HT1_A$, $5\text{-}HT1_B$, $5\text{-}HT1_D$, $5\text{-}HT1_F$, and 3 units of 5-HT2 type, e.g. $5\text{-}HT2_A$, $5\text{-}HT2_B$, 5-HT 2_C. There are two families of DA receptors, called D_1 and D_2; the D_1 family consists of D_1 & D_5 receptor subtypes and the D_2 family consists of D_2, D_3, and D_4 receptor subtypes. However, according to Stephen et al. (1997) (43), activation of the D_2 receptor is thought to contribute to the pathogenesis of migraine, and as a result D_2 antagonists might effectively manage the disorder. Hence it would be interesting to explore molecular mimics of neurotransmitters and the possibility of them activating these receptors, which could be implicated in the pathogenesis of migraine, such as tyramine. Drugs can be designed to target these receptors to bring desirable activation of these receptors, or stop the pathogenic signalling of the receptors, provided we understand the pathogenesis of the disorder and the cellular signalling mechanisms implicated in the pathogenesis process of migraine.

1.3.4 Can anti-migraine drugs act at 5-HT/DA receptors?

Hargreaves et al. (1999) (14) suggested that all triptans act as serotonergic agonists by binding to $5\text{-}HT1_B$ receptors expressed in human intracranial arteries, causing vasoconstriction and by inhibiting nociceptive transmission through an action at $5\text{-}HT1_D$ receptors expressed in the meninges and in brain stem nuclei (14). Thus, the antimigraine agents which are $5\text{-}HT1_B$ or $5\text{-}HT1_D$ agonists are effective against migraine headache pain as

they reverse the intracranial vasodilation, implicated in the pathogenesis of migraine and its associated symptoms. This might explain the link between migraine headaches (production of CGRP), and localized brain blood pressure changes as observed during CSD involving vasodilation/vasoconstriction associated with hyperaemia/oligaemia, which might have links to DA/5-HT irregularities (48). Section 1.2.2 described the current treatment modalities, in which a cocktail of drugs from anti-hypertensives to anticonvulsants being used to treat migraine. However, the MOA of these drugs suggests that it is the symptoms that are getting managed and not the disorder itself.

1.3.5 Is there any rationale behind the usage of β-blockers, anticonvulsants, NSAIDs and Vitamin-B's in migraine treatment?

As there are a wide range of triggers known to cause migraine, so are also the many treatment options available today. As mentioned before from the use of certain β-blockers, anticonvulsants, NSAIDs to vitamin B are all known to be useful in the management of migraine disorder (26, 60). This cocktail of different kinds of medication itself is an indication that there is no definitive approach for managing migraine, and that it is treated symptomatically; hence there is this complex mix of treatment pattern being practised. If all these diverse types of drugs are giving relief in the migraine patient, then there could be a common aspect of this mix of drugs contributing to the treatment success. In this context it will be interesting to explore this common factor that leads to treatment response amid such a complex mix of cocktail drug approach. Could it be the serotonin structural analogy? If such a structural analogy exists among anti-migraine drugs to a common molecule such as 5-HT or DA, and if we can establish this concept then we could explore the significance of this structural analogy in linking the resolution of the symptoms of migraine.

1.3.6 How does migraine link to 5-HT and DA systemic levels?

Even today there is no definitive answer to the question of what underlies migraine disorder or the phenomenon that triggers migraine headaches which is distinct from other headaches. Current scientific thinking points to migraine being a brain disorder linking either partly or fully to 5-HT and/or DA and their receptors, involving multiple triggering mechanisms leading to the disorder (26, 40, 55). Both DA and 5-HT have important roles in

the brain, such as the one mentioned before with 5-HT having cognitive functions in the brain, including memory and learning. Dysfunctions of the dopaminergic system, relating to the loss of dopamine-secreting neurons in the midbrain area, called the substantia nigra is associated with several important diseases of the nervous system including Parkinson's disease. Similarly, serotonergic systems are targeted by antidepressant agents to modulate 5-HT at the synapses. Hence, if DA and 5-HT are implicated in migraine, this would further add to the complexity of their role (26, 33) . The concentrations of 5-HT and DA in the synapse are important in determining their neurotransmission effects. There are several synaptic concentration control mechanisms involved to check its concentration in the synapse with the help of several presynaptic receptors and transporters (Fig. 1.9). This control mechanism ensures a fine balance of synaptic neurotransmitter concentrations. Whether interactions involving post-synaptic receptors, or pre-synaptic inhibitory receptors and/or transporters are implicated in the manifestation of the disorder is a very complex phenomenon and needs further exploration through research. A study by D'Andrea et al. (2014) on the possible role of tryptophan (TRP) metabolism in chronic migraine (CM) and chronic tension-type headache (CTTH) found that plasma levels of tryptamine (TRY) were significantly lower in CM ($p < 0.001$) with respect to the control (61). TRP is a common AA precursor to both 5-HT & TRY, the same way tyrosine is a common AA precursor to tyramine and DA. Also, it is interesting to note that TRY can mimic 5-HT, as it can activate 5-HTRs (62), and the same can be hypothesised about tyramine mimicking DA at DA receptors. Tyramine and TRY are both biogenic amines formed from their AA precursors tyrosine and tryptophan respectively. In the above study, 5-HT was undetectable in the plasma of almost all 73 samples (from 73 patients enrolled) tested (61). Their results support the hypothesis that TRP metabolism is altered in CM patients, leading to a reduction in plasma level of TRY. Also, interestingly TRY is known to modulate the functions of the pain matrix (a part of CNS active during pain sensation and is demonstrated with an imaging technique) involving the serotonergic system. This may affect modulation of incoming nociceptive inputs from the trigeminal endings and posterior horns of the spinal cord and suggest that these biochemical abnormalities play a role in the pathogenicity of CM (63).

Another study on dopamine hypersensitivity in migraine published in neurology 1997 (43), found that 35 migraineurs tested all had symptoms due to postsynaptic dopamine receptors activation (i.e., nausea and vomiting) and a lower threshold for DA receptor activation. In another study, Danish researchers used MRI (magnetic resonance imaging) scans of 19 women with migraine without aura and confirmed a significant (p=0·001) intracranial vasodilation observed during migraine attacks, compared to extra- cranial vasodilation. This indicates that future treatments need to focus on the peripheral and central pain pathways rather than simple vasodilation (14). It is also noticed that, 5-HT deficiency may result in chronic pain, sleep disturbances, anxiety, depression and a propensity to overeat (46). Low 5-HT levels are found in migraineurs and they suffer with increased frequency of depression, irritable bowel syndrome, as well as other chronic pain syndromes (46). Serotonin is important in the maintenance of extra cranial vasoconstriction and its changes in the levels in a person's brain are found to alter their mood as well (46). Also, it is noteworthy that several people are treated with antidepressants to help with migraine relief. Hence the role of 5-HT in the pain pathway is an interesting topic for research, as it seems 5-HT has a role in inhibiting nociceptive signals.

In the body, approximately 90% of 5-HT is found in stomach, and 10% in blood platelets (46), indicating both localised role and a hormonal messenger role. Hormonal headache might also have a relationship to 5-HT, since the levels of 5-HT appear to be linked to oestrogen levels (46). Migraine headache, as mentioned before, is thought to be associated with dilatation of cranial blood vessels, particularly those in the dura mater, and an accompanying localized sterile inflammatory response initiates the nociceptive signals to the brain. Both DA and 5-HT are known to play a role, via their respective receptors, in modulating the smooth muscle movements, such as contraction and relaxation of blood vessels, resulting in vasodilation and vasoconstriction via the receptor mediated cellular signalling mechanisms. Sumatriptan is a highly selective 5-HT1 receptor agonist which selectively constricts cranial blood vessels (64). Feniuk et al. (1992) also described the association of migraine headache with dilatation of cranial blood vessels in the dura mater, and sumatriptan by acting as a 5- HT1 receptor agonist, selectively constricts cranial blood

vessels and its high efficacy in migraine supports the fact that dilatation of intracranial blood vessels causes pain in migraine attacks and vasoconstriction can relieve the pain (13). A study by Ferrari et al. (1989) reported that migraineurs had plasma 5-HT levels substantially higher during attack periods and the 5-HT metabolite: 5-HIAA levels, lower during attacks compared to healthy controls (63). Thus, it can be hypothesized that systemic 5-HT metabolism is enhanced in migraineurs during non-attack periods and substantially decreased during attacks, due to lower enzymatic degradation (63).

1.3.6.1 Triptans in the treatment of migraine

Triptans are (tryptamine based) commonly used anti-migraine drugs, known by their generic names such as, sumatriptan, naratriptan, rizatriptan, eletriptan, avitriptan, zolmitriptan, almotriptan , frovatriptan, and donitriptan. It is interesting to note that Tryptamines are structurally analogous to tryptophan, an essential dietary amino acid (Fig: 1.12) from which 5-HT is biosynthesized. It is well known that amino acids, which are structurally related, can have similar functional properties. Hence triptans which are structurally related to 5-HT, may act like 5-HT at their receptors, especially in the absence of 5-HT thus correcting the systemic imbalance, and providing symptomatic relief when used as anti-migraine drugs. However, for this effect to happen they may need to cross the BBB, which means the response will be poor if adequate drug concentration is not reached at the CNS fluid. However, there is also a wide range of non-triptan based anti-migraine drugs being used, as symptomatic treatment options, including beta blockers, calcium channel antagonists, antidepressants, nonsteroidal anti-inflammatory drugs (NSAIDs), anticonvulsants and vitamin B's. Though the anti-migraine drugs are effective in treatment, their exact mode of action in relieving the disorder is still not clearly understood. Pharmaceutical medicines article (2015) (26) by authors Brinsden and Shaw are having similar work on anti-migraine drugs, where they have reported structural similarity between anti-migraine drugs and neurotransmitters DA/5-HT.

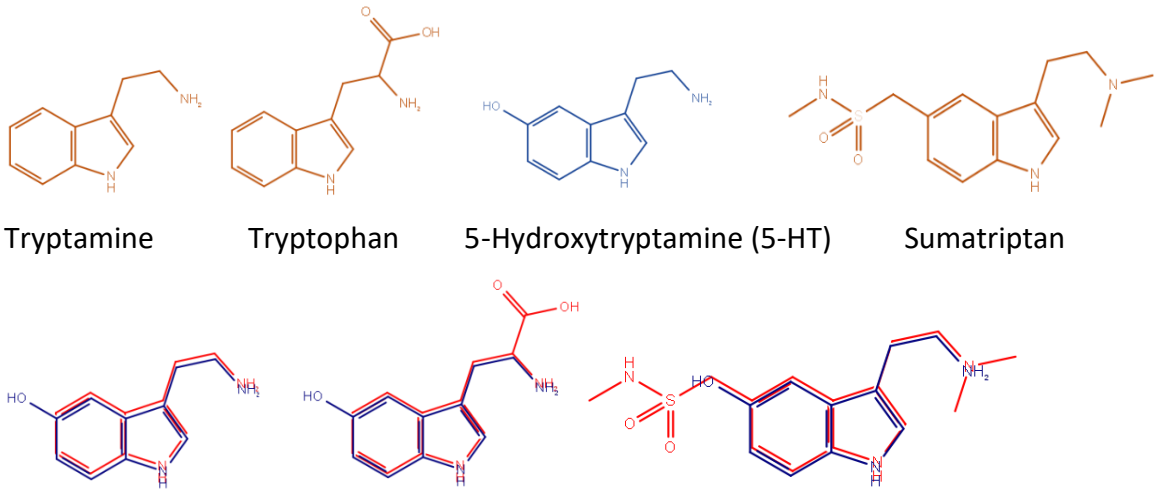


Figure 1.12 Above structural overlay of 5-HT (in blue) over (the other molecules in red) tryptamine, tryptophan, and sumatriptan, depicting structural analogy of neurotransmitter 5-HT to anti-migraine drug sumatriptan. Hence, we can assume that systemic availability of tryptamine and tryptophan indicate increased availability of systemic 5-HT as they can act as precursors and structural analogy of anti-migraine drugs to 5-HT indicate the possibility of mimicking 5-HT by lookalike molecules in the biological systems, however whether they can cross the BBB adequately is still not clear.

TRY is found in trace amounts (not significant enough to be detected) in mammalian brain, has structural analogy to TRP and 5-HT, when compared to their molecular structures (Figure 1.12), and they are all structurally analogous to antimigraine drugs such as triptans, e.g., sumatriptan. Triptans are well known tryptamine based anti-migraine drugs, and that when we study their molecular structures, all triptans share this common tryptamine molecular structure in them, hence this also suggests that there is a high possibility of mimicking 5-HT (as discussed previously) by the triptans, due to this commonality of their structure. Hence it cannot be a coincidence that all anti-migraine drugs which are effective in treating migraine, share a structurally common molecule such as 5-HT, as they are found to be activating 5-HT receptors which could be a contributing factor to their drug efficacy. As discussed earlier, 5-HT aids in the vasoconstriction of intracranial arterial blood vessels, which provides relief to migraine symptoms and DA could most likely be involved in the vasodilation, which needs further investigation. From the above discussions it can be logically concluded that 5-HT and DA has a role in the development & management of migraine, as we could assume lack of activation of serotonergic system is not able to reverse the vasodilation of intracranial arterial blood vessels, which is causing inflammatory response and pain symptoms in migraine. Hence both dopaminergic and serotonergic system needs further research in understanding their contribution to this

vasodilation (by DA/NE), subsequent pathogenic response leading to migraine and the role of 5-HT in vasoconstriction.

1.4 Understanding the pathogenesis and nociception in migraine

Current understanding of migraine suggests that, the primary dysfunction begins within the CNS, which then leads to changes in the contractility of blood vessel walls causing vasodilation of the intracranial meningeal structures, stimulating a sterile inflammatory response and subsequent symptoms of migraine. Do the current anti-migraine drugs good enough to penetrate the CNS and target this primary disfunction? Not necessarily and that explains the low drug efficacy in migraine treatment. It is believed that genetically predisposing factors could be implicated in the pathogenesis of this neurovascular disorder, with abnormalities at the cellular levels, responsible for lowering the threshold to migraine specific trigger factors, in the brain of a migraineur compared to a normal individual. However, the exact nature of the CNS dysfunction leading to CSD like phenomena and activation of the pain control centres in the brain, needs further investigation (14).

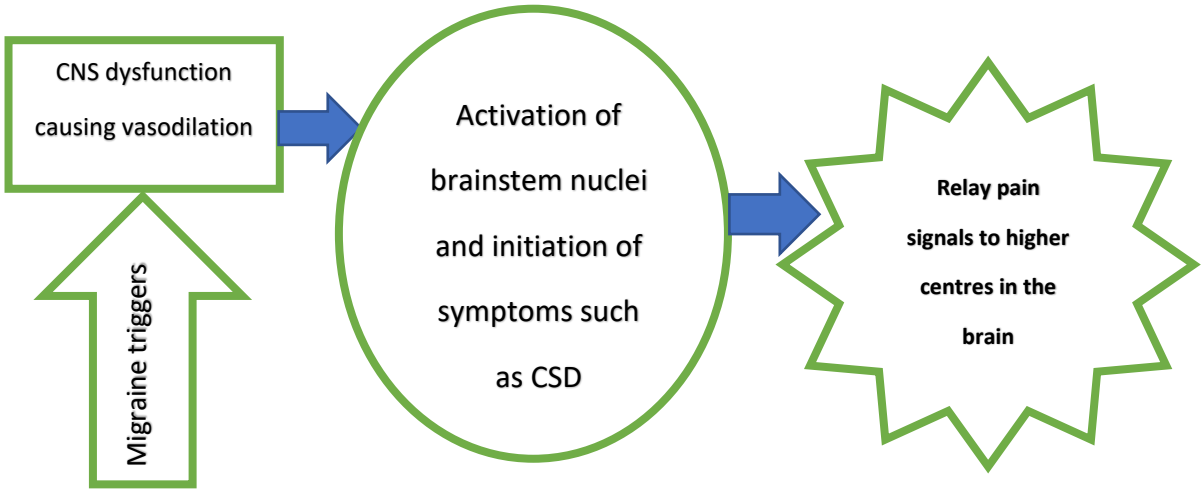


Figure 1.13 An Illustration to summarise the understanding of pathogenesis in migraine.

Based on the current scientific understandings, a key mechanism underlying the generation of headache pain associated with migraine are, the vasodilatation of intracranial & extracerebral blood vessels and a subsequent stimulation of trigeminal sensory nervous pain pathways involving the brain stem nuclei.
The network of trigeminal nerves and the associated intracranial blood vessels collectively known as trigemino-vascular system; its activation is thought to be responsible for the release of vasoactive sensory neuropeptides, such as CGRP, substance P etc. which in turn activates the brainstem nuclei causing increase in the pain response during a migraine attack (figure 1.13). The activated brainstem trigeminal sensory nuclei relay the pain signals to higher centres in brain where headache pain is perceived (14).

1.4.1 How anti-migraine agents resolve pain in migraine?

The anti-migraine agents such as triptans can act as serotonergic agonists at 5-HT1B receptors expressed in human intracranial arteries, resulting in vasoconstriction and inhibition of nociceptive transmission through an action at 5-HT1D receptors on peripheral nerve terminals in the meninges and central terminals in brain stem sensory nuclei (14). These three complementary sites of action i.e., 5-HT1B receptors on the intracranial arteries, together with 5-HT1D receptors on meningeal nerves and in brain stem nuclei (Fig.1.16) underlie the clinical effectiveness of triptans against migraine headache pain and its associated symptoms (14).

1.4.2 Brain stem nuclei as pain modulation centres

Meninges in the extracerebral region have serotonergic neurons, innervating the meningeal blood vessels, which act as afferents to pass impulses via the trigeminal and pterygopalatine ganglions from the brain nuclei as in Figure 1.15. This network may be communicating the afferent nociceptive information to the brain nuclei, where the nociceptive impulses are further modified before passing on to the higher centres in the brain. In the brain stem nuclei, such as raphe nuclei and locus ceruleus are known to have high concentrations of 5-HT and DA respectively, indicate their potential role in modulating nociceptive impulses.

1.4.3 Monoaminergic system in brain nuclei

Migraine might involve dysfunctions in the modulation of sensory inputs in the brain-stem pathways. And we may assume that monoamines such as, 5-HT, DA & NE have a role in modulating theses inputs via the raphe nuclei and the locus ceruleus (Fig.1.15 & 1.18). The trigeminovascular input from the afferent nerves innervating meningeal blood vessels pass through several nerve structures like, the trigeminal ganglion, second order neurons in the trigeminocervical complex, the quintothalamic tract, and then branch out in the brain stem, and synapses with neurons in the thalamus (40). There are several nerve connections between neurons in the pons in the superior salivatory nucleus, parasympathetic ganglia innervated by nerves from facial region. This trigeminal ganglion network may be expressed more strongly in patients with cluster headache and may be highly active in migraine, this may explain the aura symptoms associated with migraine as explained in section 1.1.2, such as sensory aura, aphasic aura, motor aura etc.

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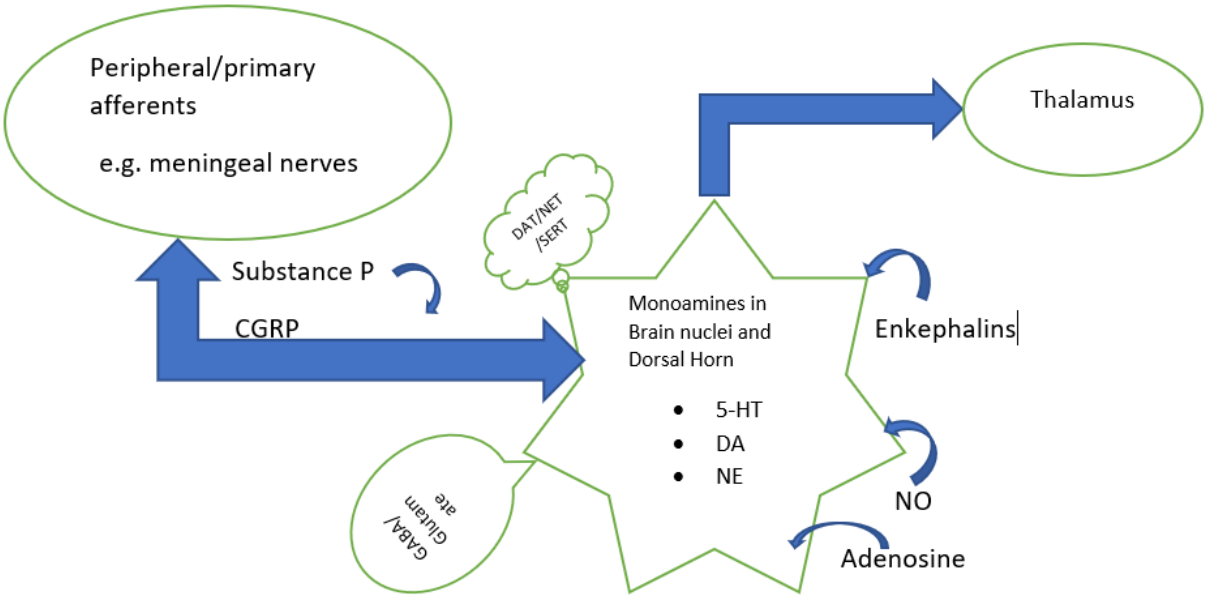


Figure 1.14 Possible pathways of nociceptive impulses in migraine.
Possible transmission of nociceptive impulses, their modulation, and processing pathway in migraine. Certain proteins at neuro junctions can control local concentrations of monoamines are DAT-Dopamine Transporter; NET- Norepinephrine Transporter; SERT - Serotonin Reuptake Transporter, some neurotransmitters that can accentuate or inhibit the impulses activated by monoamines are glutamate, GABA etc., neuropeptides that can activate nociception in brain stem are e.g. substance P and CGRP, certain chemicals that can reduce or increase inflammation such as enkephalins, NO etc.

Brain imaging studies suggest that important modulations of the trigemino-vascular nociceptive inputs come from the dorsal raphe nucleus (which has high presence of 5-HTR), locus ceruleus (has high DA concentrations), and nucleus raphe magnus (Fig.1.15) (40). The migraine pain pathway (Figure 1.14) encompasses the nociceptive afferents from the meningeal blood vessels, neurons of the dorsal horn, surrounding neurons of dorsal root ganglion and a distributed supraspinal network that modulates relay and processing of nociceptive inputs to the dorsal horn.

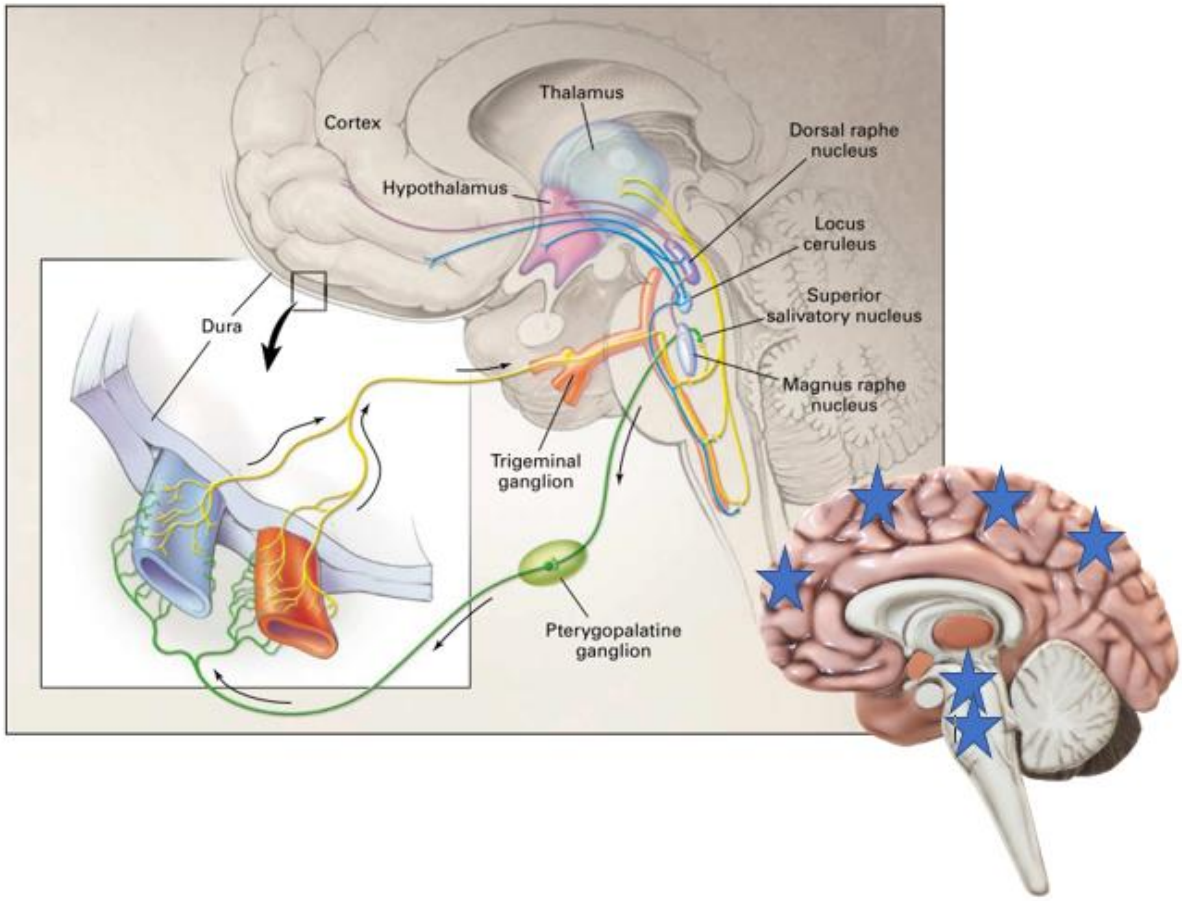


Figure 1.15- Trigemino-vascular network components in brain stem (Images borrowed from Goadsby et al. article from New England Journal of medicine 2002 (40) with copy right permissions to reproduce in academic work, inset brain model downloaded from MS word online pictures which doesn't need copy rights permissions). Additional brain model in the inset, displays regions highlighted with blue stars to depict the likely distribution of 5-HTRs. Extracerebral regions including meninges are densely innervated by neurovascular network which act as afferents in transmitting nociceptive impulses upon non-sterile inflammation due to increased blood flow during a migraine attack. Brainstem in the intracranial region has both serotonergic as well as dopaminergic neurons via dorsal raphe nuclei and locus ceruleus. Trigeminal ganglion mediates connections between extracerebral, intra-cranial neuron pathways to the trigeminal nuclei, especially when all the important brain nuclei are centred in the brain stem.

The glutamatergic inputs as illustrated in Figure 1.14 can enhance the nociceptive signals from sensory afferents to several laminae of the spinal dorsal horn. Neuropeptides, such as substance P and CGRP can be released by sensory afferents in brain (including meninges) and thus this in turn can sensitise dorsal horn neurons resulting in neuropathic pain such as migraine (Fig.1.14) (15).

As per Benarroch et al. (2008) nerve impulses from spinal cord areas such as Lamina I and lamina V, comprising the spinothalamic tract which conveys nociceptive inputs to the hypothalamus, amygdala, periaqueductal gray (PAG), and rostral ventromedial medulla (RVMM) (15). Lamina II contains inhibitory GABAergic interneurons that utilize glycine, or opioids such as enkephalins, and provide impulses to communicate or inhibition of nociceptive signals to the spinothalamic and spinobulbar projection neurons. 5-HT, DA and NE play a vital role in transmitting nociceptive impulses by being pro-nociceptive or anti-nociceptive at their selective receptor sub types (Fig.1.18). By being either anti-nociceptive or pro-nociceptive at their selective receptor subtypes, these neurotransmitters can modulate or control nociceptive impulses, which is crucial in activating pain sensations as illustrated in Figure 1.16. Once the pain is activated, the intensity can be accentuated by other intervening glutamatergic nerves or ameliorated by GABAergic inhibitory neurons as illustrated in Figure 1.20. This kind of emphasising bidirectional influences on pain sensation can either inhibit or facilitate transmission of nociceptive impulses at the dorsal horn area of brain. These modulatory effects are largely mediated by descending monoaminergic pathways that utilize serotonin, norepinephrine, or dopamine as illustrated in Figure 1.18.

The descending monoaminergic system processing nociceptive impulses are in the dorsal horn region of brain. Processing these impulses and their effects in the dorsal horn are complex. Monoamines may act via different subtypes of receptors between their locations at peripheral afferents like meningeal nerves and dorsal horn projection neurons and may involve other local excitatory or inhibitory interneurons such as GABAergic and glutamatergic neurons, and glial cells. Serotonin, norepinephrine, and dopamine may exert either antinociceptive or pronociceptive effects according to the type of receptor sub type

involved, site of action in the dorsal horn, brain nuclei or at peripheral afferents and crosstalk between descending monoaminergic systems including DA/NE and local neurochemical signals, including NO, adenosine and endogenous opioids such as enkephalins (Fig.1.19) (15). In addition, concentration of monoamines in the dorsal horn and brain stem are regulated via controlled release and reuptake mechanism at the synaptic clefts (15).

1.4.4 Deactivation of nociception in migraine by 5-HTRs

5-HTRs play a crucial role in migraine (Figure 1.16), as their vasoconstrictive effect can correct the inflammation at meningeal blood vessels via 5-HT_{1B} and may help reduce the blood flow in brain at the same time. 5-HT_{1D} receptors inhibit nociceptive transmission via CGRP inhibition as described in figure 1.16, however in the absence of these receptor activations, migraine symptoms may start to develop, and when an anti-migraine drug is administered it might correct the situation by mimicking the natural ligand and activate these receptors and correct the situation to relieve the symptoms.

Hence the underlying mechanism we may assume, that leads to the migraine disorder could be the 5-HT/DA dyshomeostasis, leading to hyperactivation /sensitisation of brain centres and that the triptans are able to partially correct this dyshomeostasis. Figure 1.16 illustrates the neurotransmission pathway of 5-HTRs between brain nuclei and blood vessels via trigeminal ganglion, and the communications between higher centres in brain and the dorsal horn with brain nuclei in controlling the nociception pathways.

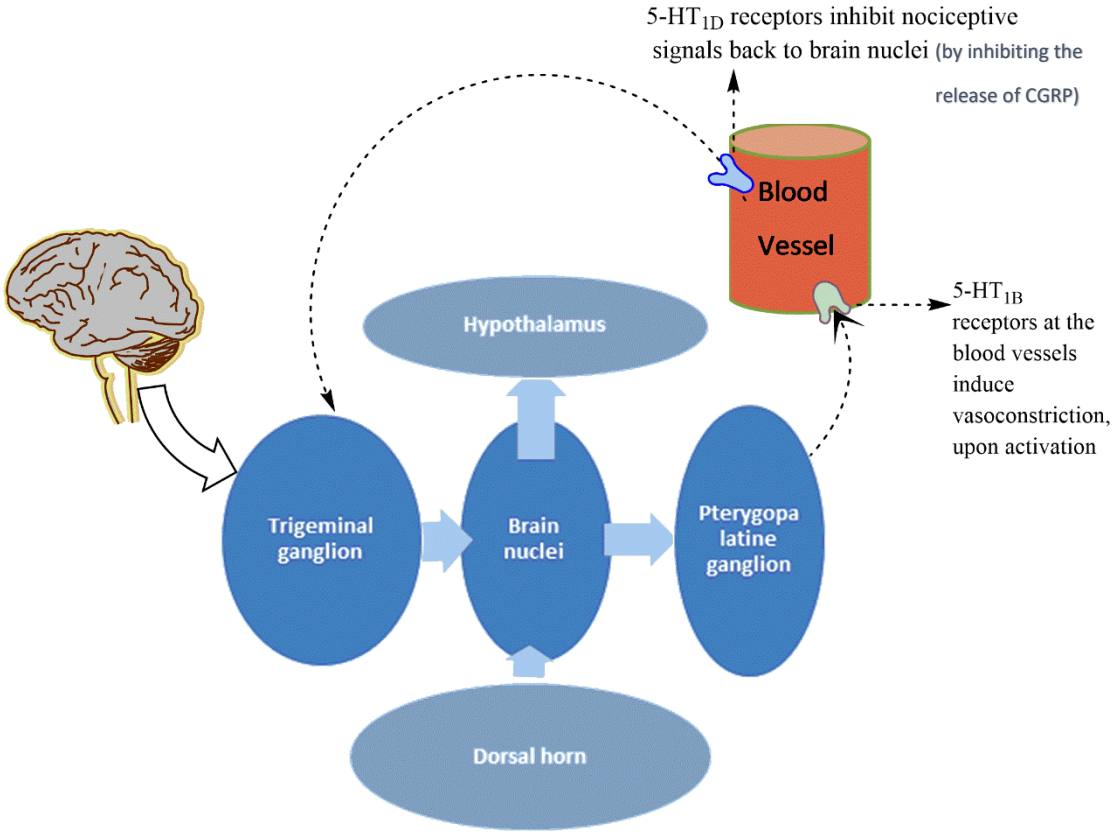


Figure 1.16 A schematic representation of the signalling pathway of neurotransmission resulting in vasoconstriction of blood vessels, due to the presumed activation of 5-HTRs by natural ligands or by anti-migraine drugs such as triptans.

1.4.5 Other predisposing factors in migraine: the role of DA lookalike- 'tyramine'

In a double-blind study (65) on the effect of tyramine in migraine, the electroencephalogram changes observed as part of the study observations supported the hypothesis that tyramine has an action on the central nervous system in some migraine patients (65). As tyramine is a biogenic amine as mentioned earlier, derived from AA tyrosine, available through our daily dietary intake and has a close structural analogy to dopamine (DA), which will be interesting to correlate their role and explore their influence due to this structural analogy.

A cross-sectional study published in The Lancet Neurology 2013 (66), has used magnetic resonance angiography of intracranial arteries in patients with spontaneous migraine without aura, suggests that migraine pain was accompanied by slight intracranial dilatation, this supports the understanding that migraine headache might originate from dilated intra cranial arteries involving neurobiological mechanisms, hence future migraine research focusing on the peripheral and central pain pathways involving trigeminal nervous system would be interesting to help further understandings on pathogenesis of migraine.

1.4.6 Genetic factors

Though there are varying theories; the specific cause of common forms of migraine headache is still unknown, however, studies on families suffering from familial hemiplegic migraine (FHM) have suggested that genetic factors may be involved (14). The occurrence and frequency of attacks in any individual will be influenced by their CNS response to migraine specific triggers (e.g. certain foods, hormone levels in blood, changes in levels of stress, reading habits and even exposure to light and sound). It has been hypothesized that genetic abnormalities result in a lowered CNS threshold of response to these specific trigger factors in migraineurs, that means even a low level of trigger can activate a response at the brain stem, or in other words the brain stem nuclei are more sensitised to triggers than in a normal person. Hence, we could assume that the trigger factors can influence as modulators of the genetic set point that predisposes to migraine, however this modulatory influence is only assumed as nothing is proven as a clear factor in influencing the response mechanism at the brain nuclei in a migraine patient. It is possible that the unusual hyper-responsivity of the brain in migraineurs, like FHM, may be a consequence of mutations in the ion channels that regulate neuronal excitability. This has given rise to the description of migraine as a channelopathy wherein irregularities in the functioning of ion channels leading to improper firing of signals from neurons. As we know from earlier section 1.2.1, that to conduct an AP via synapse, ion channels like Ca^{2+} ion channels, must allow inflow of Ca^{2+} ions to excite neurotransmitter vesicles to move towards the synapse to release the neurotransmitters to the synapse. Only then the AP is transmitted to the post synaptic neurons, via these neurotransmitters which then binds to the respective

receptors in the post synaptic neurons to pass on the impulses. The theory suggests that, due to this channelopathy, migraine specific triggers can provoke or activate CNS as explained in section 1.4.3 and lead to dysfunctions characterised by initiation of migraine specific symptoms such as experiencing sensory auras, characterised by visual disturbances, photophobia, hypersensitivity to sound, smell etc. In this context the presence of blood flow changes, such as oligaemia and hyperaemia, could be an indicative of altered CNS activation and have supported a role for CSD or activation of early symptoms of migraine (especially with aura). CNS dysfunction produced by various migraine trigger factors could be an explanation as to why some patients who present symptoms of migraine with aura and some without aura. This was supported by the observation that patients with no history of migraine developed migraine-like episodes after surgery to implant electrodes in the periaqueductal grey matter and raphe nuclei in the brain stem (14).

Another hypothesis is that the primary dysfunction in migraine occurs within the CNS leading to changes in blood vessels within pain-producing intracranial meningeal structures that give rise to headache pain. Hence, migraine is now thought to be of a neurovascular and neurobiological disorder. It has been proposed that genetic abnormalities may be responsible for altering the CNS response threshold, to migraine specific trigger factors of a migraineur compared to a normal individual (14). Though there are varying explanations to the nature of the CNS dysfunction that result in migraine, it is still not clear to point to a single cause and also that, what could trigger the CSD like phenomena and activation of nociceptive mechanism in brain stem nuclei involving dorsal horn in migraine patients need to be further investigated (14). It is generally thought that cerebral vasodilation and a consequent stimulation of surrounding trigeminal sensory nervous pain pathways is a key mechanism, as mentioned in section 1.4.3, underlay the generation of headache symptoms associated with migraine (14).

1.4.7 Role of monoamines and their receptors in migraine

The initiation of pain mechanism as explained in section 1.4.3 includes in general, the nociceptive information from peripheral nerves (afferents), a well distributed supraspinal network of neurons that modulates relay and processing of nociceptive inputs from several laminae in the dorsal horn region and central brain nuclei. Primary nociceptive afferents provide excitatory glutamatergic inputs to several laminae of the spinal dorsal horn. Some nociceptive afferents also release neuropeptides, such as substance P and CGRP (in Brain), which has a key role in the mechanisms of sensitization of dorsal horn neurons in conditions such as neuropathic pain in brain stem and possibly activating monoaminergic neurons, such as 'serotonergic', 'dopaminergic' and 'noradrenergic' neurons (Fig.1.17) in migraine. Some inhibitory interneurons that uses GABA, glycine, or opioids such as enkephalins (Fig.1.19) can provide a cross talk inhibition of monoaminergic system to the spinothalamic tract (STT) neurons (15).

As per Benarroch et al. nerve impulses from PAG to RVMM route comprises serotonergic nerves, has been classically considered the primary endogenous pain modulatory system and target of supraspinal opioid analgesia (15). However, the RVMM contains functionally heterogeneous groups of serotonergic and non-serotonergic neurons that are not only involved in pain modulation but also in control of autonomic nervous system and other homeostatic functions (15). The RVMM contains two types of cells: 'off' cells that are inhibited by noxious stimulation and excited by opioids, and 'on' cells that have an opposite pattern of response (15), i.e., the 'off' cells inhibit nociceptive transmission, whereas 'on' cells stimulate nociception via activation of mono aminergic pathway between hypothalamus and spinal cord as there are both an ascending and descending monoaminergic pathway comprising of 5-HT, NE and DA as mono amines. However, it is hypothesised that, 5-HT acting via different receptor subtypes, exerts this complex 'on' and 'off' functions on nociceptive transmission in the dorsal horn and brain nuclei. Hence the modulatory effect of 5-HT is a complex process involving several types of 5-HTRs, where in some may be activating nociception and some inhibiting nociception, which could have a crucial role in conditions like migraine and is extensively studied topic.

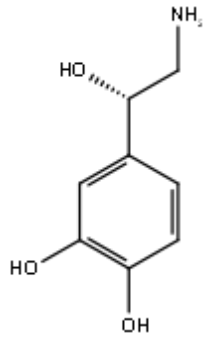
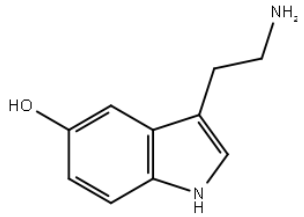
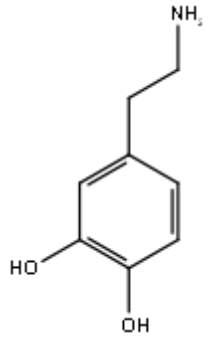
Neurotransmitter	Region in brain	Antinociceptive receptors present	Pronociceptive receptor present
Noradrenaline 	Locus ceruleus-subceruleus, A5	α_2 α_1	?
Serotonin 	RVMM, (NRM)	5-$HT_{1B/1D}$ (STT neuron)	5-HT_3, 5-HT_2
Dopamine 	A11, Locus ceruleus	D_2, D_3 (STT neuron)	D_1 (STT neuron)

Figure 1.17 List of both pronociceptive and anti-nociceptive monoaminergic receptor subtypes and their respective neurotransmitters (Table borrowed from article by Benarroch et al., Neurology 2008 (15))
In specific locations of CNS (15). A11 cells are DA specific neurons and A5 are NE specific. RVMM (rostral ventromedial medulla); NRM (nucleus raphe magnus); STT (spinothalamic tract).

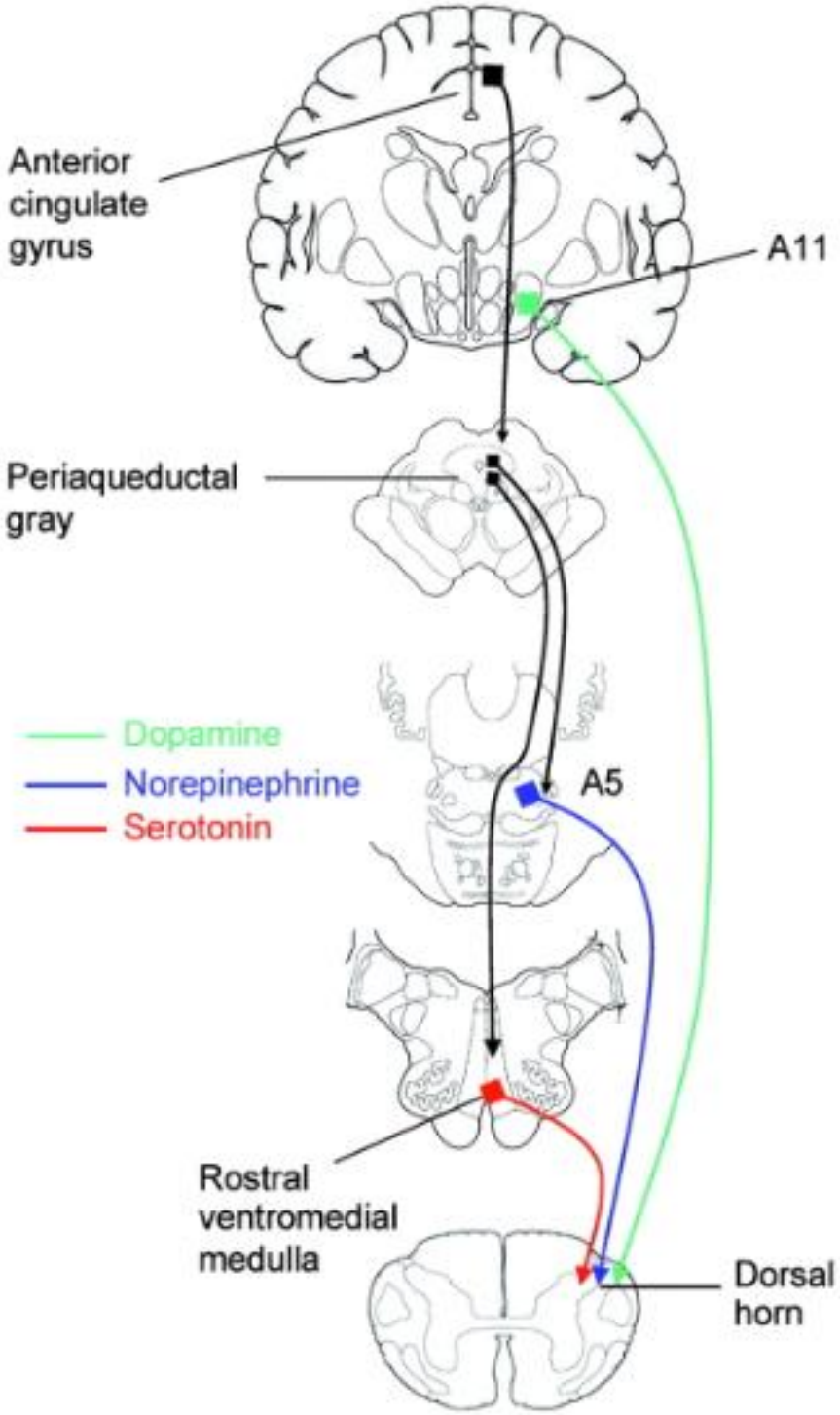


Figure 1.18-Monoaminergic modulation of pain transmission in the dorsal horn involving serotonin as illustrated by Benarroch et al. in the Journal of Neurology 2008. (Image borrowed from article by Benarroch et al., Journal of Neurology 2008).

As per Benarroch et al. (2008), activation of 5-HT1 receptors such as, 5-$HT_{1A,}$ 5-$HT_{1B,}$ & 5-HT_{1D} receptors inhibit nociceptive impulses (15) in STT by inhibiting neurotransmitter release from primary afferents probably by stopping CGRP release into synapses (Fig.1.20), however, 5-HT2 & 5-HT3 receptors exert a pronociceptive effect via the monoaminergic system (15), probably by influencing increased neurotransmitter release from primary nociceptive afferents. Thus serotonergic neurons have a dual role in pain processing; they facilitate nociceptive transmission via specific 5-HTRs and suppress the nociceptive transmission via another set of 5-HTRs and prevent neurogenic inflammation (15).
The pain matrix in the CNS, includes important areas that are active in the pain modulation process; such as cingulate gyrus, PAG, dorsolateral pontine tegmentum, and ventromedial medulla (15), exert either antinociceptive or pronociceptive effects via monoaminergic system comprising of 5-HT, NE, or DA as their primary neurotransmitters. Hence, we can assume that there is a group of neurotransmitters involved in nociception and do cross talk between each other to modulate their impulses. There are specialised neuro-cell lines that express these monoaminergic receptors in the pain matrix of the CNS, such as A11 cell lines express DA receptors, A5 cell lines express NE receptors, where they play a role in nociceptive transmission. D_2 agonists may facilitate antinociception by potentiating the effects of endogenous opioids; however, D_1 agonists facilitate nociception probably by antagonizing D_2 or opioid
receptors. The effects of DA on spinal nociception may depend on its local concentration, as low levels may activate the antinociceptive D_2 type receptors, whereas high levels activate the pro-nociceptive D_1 receptors (15). Which is interesting as high levels of DA can facilitate inflammation and low levels inhibit the same effect; which may have a role in the activation of symptoms in migraine, and this is in line with earlier section 1.1.3 on molecular mimics of DA from dietary AAs affecting its systemic level. Hence this supports our assumption that molecular mimics may have a role in triggering migraine response from brain as high levels of DA activate the pro-nociceptive D_1 receptors.

The overall balance between inhibitory and excitatory supraspinal signals mediated by monoamines provide the basis for total modulation of pain sensation also the same area of brain is involved in other roles of emotional attributes such as motivation, fear, anxiety &

other behavioural variables and has an important role in the mechanisms of inflammatory and neuropathic pain (15).

In the dorsal horn, monoamines such as, 5-HT, NE & DA exert an antinociceptive action primarily by reducing neurotransmitter release from primary afferents; these effects are mediated by presynaptic 5-HT_{1B}, D_2 & D_3 receptors, and postsynaptic inhibition of STT neurons. In contrast, serotonin acting via postsynaptic 5-HT2, both pre-and postsynaptic 5-HT3 receptors, and DA, acting via D_1 receptors, may have a pronociceptive effect. NE may elicit antinociception indirectly via GABAergic neurons probably via activating the release of GABA to inhibit pronociceptive signalling. Presynaptic reuptake mechanisms via transporter proteins and presynaptic neurotransmission inhibitory effects via GABA ergic neurons regulate the local levels of monoamines and thus their effects on different targets in the dorsal horn is a monoaminergic level dependant controlled process based on the above discussed facts (Figure 1.20) (15).

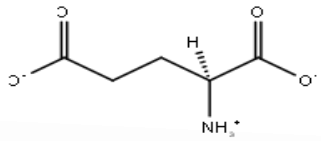
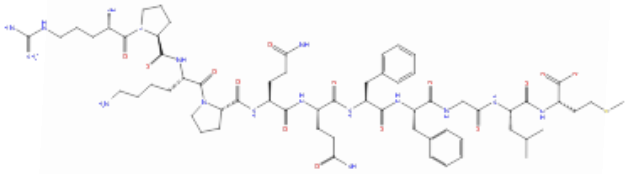
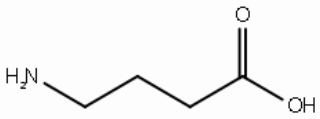
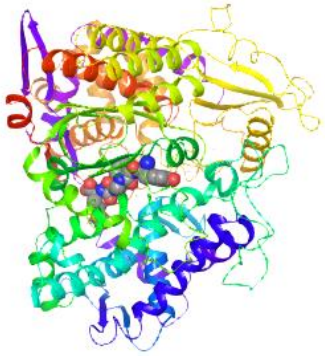
Target	Neurotransmitter/ Neuropeptide
Primary nociceptive afferents	Glutamate  substance P (composed of 11 AA)  CGRP (Composed of 37 AA)
Spinothalamic neurons	Glutamate (refer structure above) CGRP (Below in complex with its receptor) 
Excitatory interneurons	Glutamate (refer structure above)
Inhibitory interneurons	GABA  Enkephalin (shown below in complex with its receptor) 

Figure 1.19- Other biochemicals that contribute to nociception in migraine with their respective target neuron sites (Table partially borrowed from article by Benarroch et al., Neurology 2008 (15))
biochemicals involved in nociceptive neurotransmission at their specific target neurons, may influence or activate monoamines which transmit nociceptive signals, which act via different subtypes of receptors located at the primary nociceptive afferents, dorsal horn projection neurons, local excitatory or inhibitory interneurons, and glial cells (15). (AA: Amino Acid)

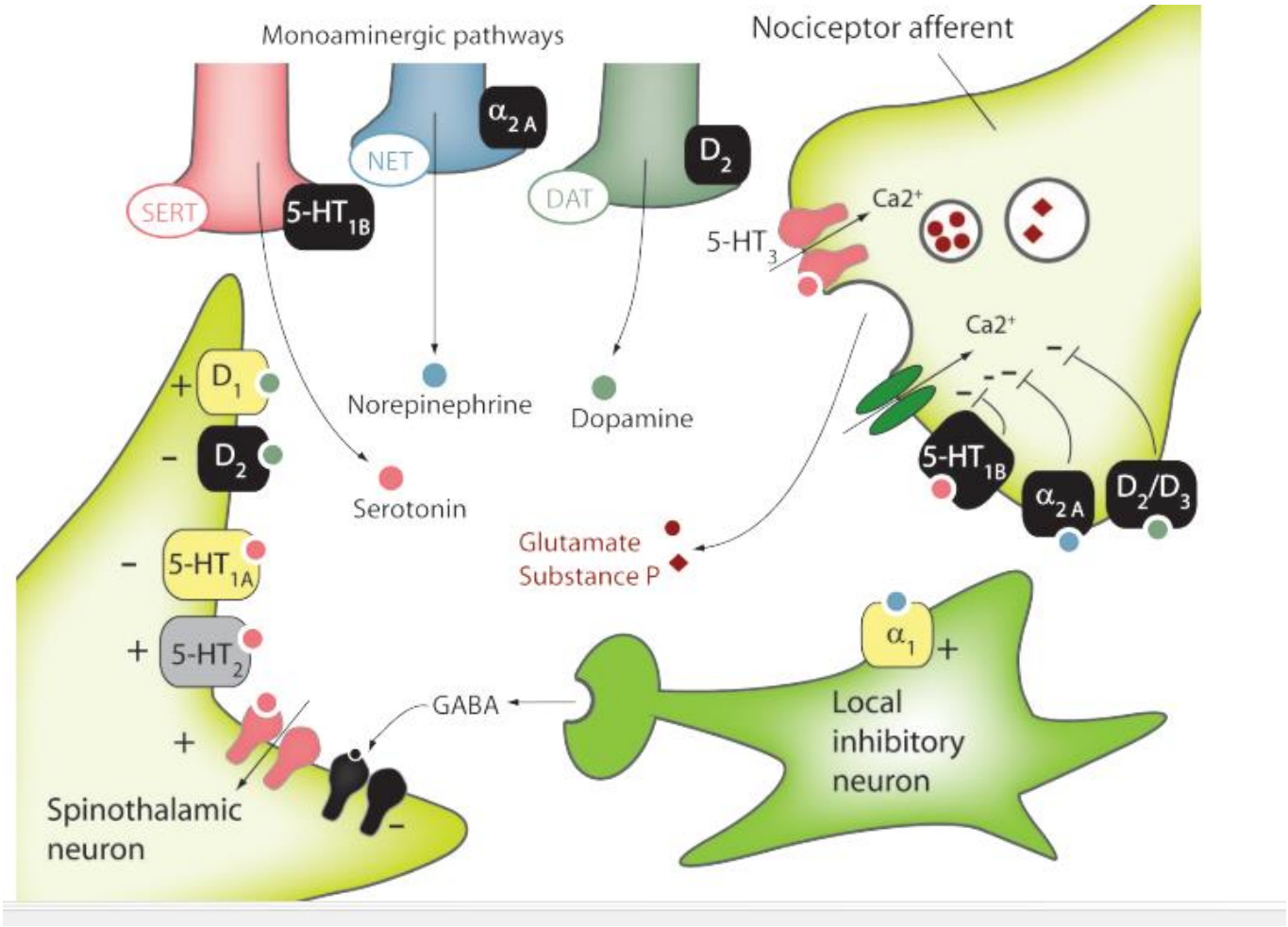


Figure 1.20 Receptor-neurotransmitter activation mechanisms at synapses in dorsal horn (Image borrowed from article by Benarroch et al., Neurology 2008 (15))
Potential targets and receptor mechanisms mediating the pain modulatory effects of monoamines in the dorsal horn as illustrated by Benarroch et al in the Journal of Neurology 2008. Neurons involving 5-HT can either inhibit nociceptive signals or stimulate signals resulting in pain modulation e.g. enkephalin containing neurons.

1.4.8 The role of vasoactive biochemicals and neuropeptides in migraine

Primary afferents when activated due to a stimulus, can release various neuropeptides and biochemicals along with neurotransmitters into the synaptic junctions depending on its site of origin, which then activates the postsynaptic neurons via neurotransmitters. Presence of neuropeptides or vasoactive biochemicals can enhance the postsynaptic signalling, which could be an inhibitory or an agonistic response based on the type of receptors the

neurotransmitter activates. If it activates an inhibitory neurotransmitter receptor response, such as an antinociceptive receptor response, then the inhibitory response is conducted down the line and this response can be further enhanced by a glutamatergic neuron and the response can be reduced by a GABA-ergic neuron. Hence the presence of a neuropeptide can aggravate the transmission of signals at glutamatergic neurons, which could also be a pronociceptive receptor response, which is probably implicated in migraine.

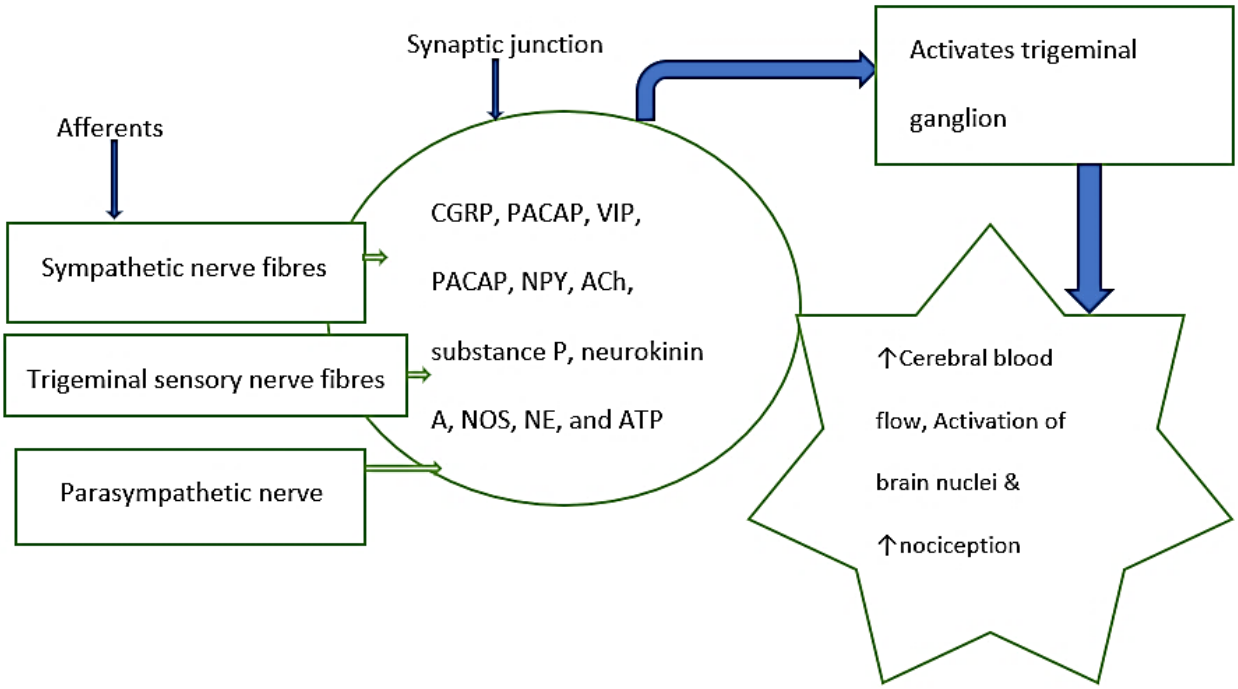


Figure 1.21 Vasoactive neuropeptides and biochemicals in migraine and the possible nociceptive pathways
Vasoactive biochemicals and neuropeptides probably influencing migraine response are: Calcitonin Gene-Related Peptide (CGRP), Pituitary Adenylate-Cyclase Activating Peptide (PACAP), as well as Nitric Oxide Synthase (NOS), Adenosine Triphosphate (ATP) and Norepinephrine (NE) Vasoactive Intestinal Peptide (VIP), Neuropeptide Y (NPY) and Acetylcholine (ACh) (19).

These vasoactive peptides activate the trigeminal ganglion (Figure 1.21), which results in increased extracranial blood flow and stimulation of the Superior Sagittal Sinus (SSS), which leads to increased cerebral blood flow (19). As mentioned before, in humans CGRP is elevated during the headache phase of severe and untreated migraine and cluster headache. Trigeminovascular nociceptive activation can contribute to the pain that is experienced in migraine, and that CGRP release is a major contributor to this. Imaging studies conducted in migraine have indicated that areas of the brainstem are active during migraine and involves the hypothalamus and thalamus (19). Hence brainstem areas are at the heart of the pain processing pathway and are therefore likely to be at the heart of migraine pathophysiology. What causes the activation of the brainstem and the trigeminovascular pathways is still debated, and that fundamentally drives migraine pathophysiology (19).

1.4.9 Nitric oxide (NO) donors and neuropeptide vasodilators as triggers of migraine

NO donors, such as nitro-glycerine (consumed as a medication) and other potent vasodilators including CGRP, Pituitary Adenylate-Cyclase Activating Peptide (PACAP), Vasoactive Intestinal Peptide (VIP), and prostaglandin I_2, are all likely to cause an immediate headache and vasodilation of intra cranial blood vessels in patients and control volunteers in trials tested (19). As noted in previous sections, intra cranial vasodilation is known to be a key mechanism triggering migraine and headache symptoms.
Hence all biochemical vasodilators are able to trigger migraine response in migraineurs, as they have the potential to activate the trigeminal nervous system (19). This is because, these biochemicals induce nociceptive responses from the afferent meningeal nerves, activate trigeminal nuclei in migraine patients. Firing of these trigeminal nuclei, induce inflammatory response and referred to the higher order centres, such as thalamus and hypothalamus and this might explain the throbbing pain and other symptoms that is experienced in migraine (19).

1.4.10 Neurobiological events associated with migraine symptoms

Aura phase, as described in previous section 1.1.2, cortical spreading depression (CSD) and focal neurological symptoms characterized by visual, sensory and/or dysphasic speech symptoms in migraine are linking to the activation of the trigeminal vascular system. Human studies have demonstrated a biphasic change in cortical blood flow during aura and leading to an initial brief hyperaemia followed by a more prolonged oligemic spreading across the cortex, like the phenomenon described earlier as CSD, which is understood to be a wave of neuronal depolarization, induced by chemical or physical stimuli, that spreads across the cortex at similar speed to aura (19).

1.5 Known signalling pathways of serotonin

1.5.1 Serotonin and its receptors

5-HT which is commonly known as serotonin, or 5-hydroxytryptamine or as simply as a general monoamine, acts in multiple roles as a biogenic molecule such as a hormone, a neurotransmitter, a neuromodulator, a mitogen and possibly many more roles unexplored (47). Dysfunctions in serotonin system such as its receptors and Serotonin Reuptake Transporters (SERT) are associated with irregularities of insulin sensitivity, appetite, sleep, organ development, behavioural disorders such as anxiety. Degeneration of serotonergic neurons and loss of serotonin receptors are implicated in many neuropsychological conditions such as, schizophrenia, clinical depression, anxiety disorders, Alzheimer's and Parkinson's disease (47). Hence serotonergic system is increasingly targeted for drug development, such as Selective Serotonin Reuptake Inhibitors (SSRI's) are one such example of being increasingly used in most of the neuropsychiatry disorders. SSRI's block SERTs there by increases the availability of extracellular 5-HT and increases their chance for binding to 5-HTRs (47). Increased 5-HT transmission is associated with increased adult neurogenesis in animal models which aids in neurodegenerative disorders such as Parkinson's, Alzheimer's and conditions of depression. 5-HTRs are thought to have influence on glucose homeostasis and thereby 5-HT regulates food intake, energy metabolism, stress responses and blood pressure via modulation of neuroendocrine and the autonomic nervous system pathways (47).

1.5.2 Early discovery of Serotonin

During late 1930s, Vittorio Erspamer described 5-HT as an enteramine which is present in gastric mucosa and enterochromaffin extracts and can induce smooth muscle contractions. Later Maurice Rapport of Irvine page's lab isolated 5-HT and reported, it can induce blood vessel constriction. In the early 1950s, 5-HT was recognised as having a neurotransmitter property and that it was found to mimic ionotropic & chronotropic effects of acetylcholine antagonists in heart muscles and induced relaxation of certain type of muscles. Later it was suggested that 5-HT could be involved in neuronal signalling as well as in regulating other neurotransmitters. By early 1960s the source of 5-HT in brain was identified as small

clusters of serotonergic neurons within the raphe area of the brain now known as brain nuclei (56).

1.5.3 Early discovery of 5-HTRs

About 10 years after serotonin was discovered, its receptors were identified in a study on gastric tissues in guinea pig, then in late 1970s, Peroutka and Snyder identified 5-HT in rat brain cortex tissues and were designated as 5-HT_1R and 5-HT_2R (56). Later Cerrito and Raiteri provided the first evidence of 5-HTR localisation in presynaptic neurons, which suggested the complexity of serotonergic system (67). Then by early 1990s to 2008, all subsequent families of 5-HTRs totalling to 7 members were identified as a group of GPCRs (G protein coupled receptors) and to date all the 14 subtypes excluding 5-HT_3R, which is a cation channel, are categorised as a group of GPCRs (56)

1.5.4 5-HTR signalling mechanism

Studies with partial reduction in 'brain 5-HT', were found to have reduced brain growth and cortex formation suggesting its role in multiple physiological functions (56). Imbalance in 5-HT signalling pathways are implicated in various pathophysiological and neurodegenerative disorders such as schizophrenia, Alzheimer's disease, anxiety and depression (56, 68). We presently lack a complete understanding of the downstream molecular signalling mechanisms involving 5-HT in CNS; however, based on various reported evidences and events involving various subtypes of 5-HTRs in other parts of the body and their downstream effector molecules in different areas of brain, I will provide an overview of the possible signalling and the different downstream molecular mechanisms in neuronal disorders. However, there will be limitations to this understanding as the signalling will differ according to the cell type and the response from cell to other cell types such as the cells in gastric region and brain.

Considering 5-HTRs potential, in influencing wide-range of physiological functions throughout the body, they have become the prominent targets for pharmacological therapies, such as antidepressants, antipsychotics, migraine therapies, appetite suppressants, and drugs for the treatment of gastrointestinal disorders. The known abused drugs, such as the serotonergic hallucinogens e.g., lysergic acid diethylamide (LSD), DMT (N, N-dimethyltryptamine) and psilocybin (figure 1.22) are all known to act directly on the 5-HTRs in CNS. These drugs by acting as ligands, at 5-HTRs are inducing signalling pathways

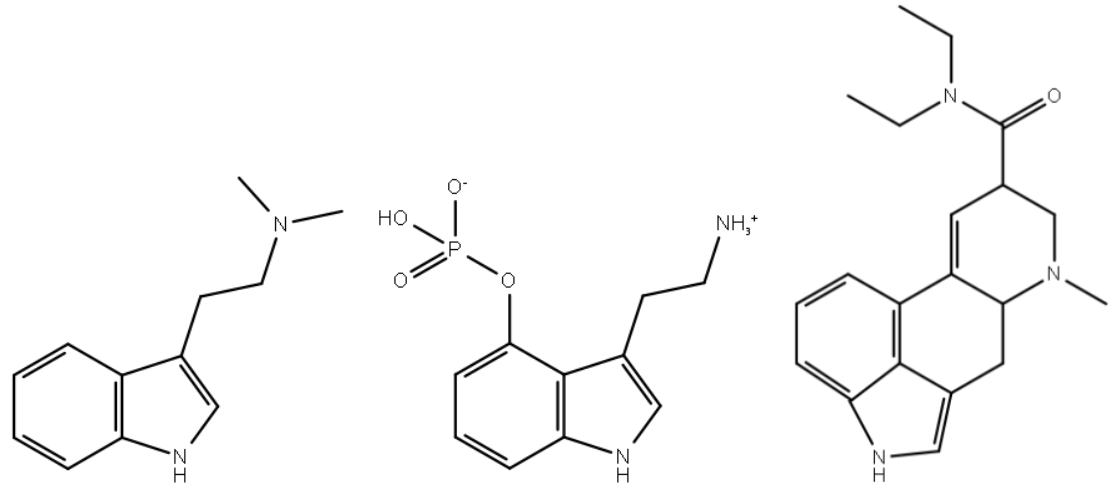
by recruiting secondary molecules specific for the ligand type indicating a functional selectivity possibility as each ligand differ in the extent to which they stabilise a receptor conformation and the selection of a signalling pathway is dependent on the receptor conformation changes induced by a ligand and the stability of this conformation (69). 5-HTRs are also indirect targets of psychostimulants such as amphetamines and cocaine (69). 5-HT activates the 5-HTRs which in turn exposes its coupled Guanine nucleotide binding proteins (G proteins) via conformational change, which is bound to GDP (Guanosine diphosphate), gets phosphorylated to GTP which switches on the protein for initiating a signalling pathway. The heterotrimeric G proteins have 3 components viz, G_α, G_β & G_Υ and in turn each of these 3 have several subtypes, such as inhibitory G_α subunit, denoted by G_i, stimulatory G_α subunit denoted by Gs and so on. G_q & G_o are also such examples of other types of G_α subunits, and it is important to note that each type can initiate a unique pathway. Also, the fact that there are several subtypes of 5-HTRs as discussed earlier, which are coupled to G proteins and each 5-HTRs have a unique mechanism of signalling adds to the complexity of their pathways. However, all these signalling mechanisms, in general can be grouped to 2 main pathways for simplicity as G protein mediated and β-arrestin mediated pathways.

An agonist ligand upon binding to a GPCR can stimulate the activation of multiple signalling pathways, in 2 separate ways. They are G protein-mediated pathway and β-arrestin-mediated or G protein independent pathway. Also, it is interesting to note that, the extent of activation of each of the downstream pathway depends upon the ligand, as it induces a unique receptor conformation, with some ligands, such as 5-HT lookalike drugs, promoting activation of one pathway over another. This concept of an agonist selectively stabilizing a special receptor conformation, causing the receptor to recruit secondary signalling molecules preferentially over another expected pathway to which it is coupled, has been termed functional selectivity or biased agonism (52, 56, 57, 70, 71). Some 5-HTRs coupled to G protein can recruit multiple signalling molecules via either G protein mediated way or β-arrestin way without preferring any specific pathway. However, some 5-HTRs (e.g. 5-$HT2_B$) can preferentially recruit signalling molecules such as β-arrestin over G protein molecules, there by masking the G protein mediated signalling, referred to as a

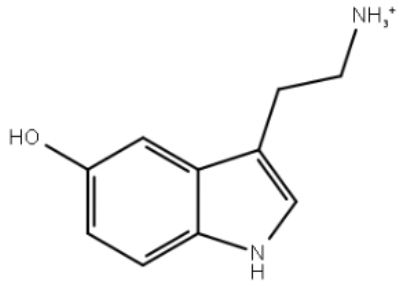
property called functional selectivity (72). This functional selectivity is entirely dependent on the unique conformational change induced by a ligand on the receptor and the stability of this conformational change leads to different or additional residue exposure for phosphorylation (69).

1.5.5 5-HTR signalling via β-arrestin mediated pathway

It is now known that psychedelic drugs such as LSD (lysergic acid diethylamide) which are known to show their hallucinogenic properties are found to act via 5-HTRs (54), and they differ in recruiting the downstream signalling molecules by selectively choosing β-arrestin (most probably β-arrestin 1 over β-arrestin 2) pathway V/s the natural ligand 5-HT which seems to recruit in a non-preferential way either G protein or β-arrestin (seems β-arrestin 2 over β-arrestin 1) and that explains the hallucinogenic property of LSD over its natural



DMT Psilocybin LSD



5-HT

Figure 1.22 Examples of psychedelic drugs with known serotonergic activity. Careful study of their structures reveal a structural analogy to 5-HT, which might have lead to their serotonergic activity.

ligand (69, 72). It is interesting to note that 5-HT can activate extracellular signal–regulated kinase (ERK), or alternately known as mitogen activated protein kinase (MAPK) mediated pathway via G protein recruitment. However it is now not clear either via β-arrestin 2 or β-arrestin 1 recruitment (both generally referred to as β-arrestin), although both seem to have a unique pathway and if the receptor stabilises for a longer time like in LSD via β-arrestin 2 (69), the signalling effects stay longer, and it deactivates the signalling via G protein pathway, which contributes to its hallucinogenic property (72). Unlike with natural ligand, where β-arrestin (β-AR) recruitment quickly internalises the receptor, however in some cases this complex is still able to activate ERK/MAPK even during and after internalisation (Fig. 1.23), for some time longer than expected (71, 73).

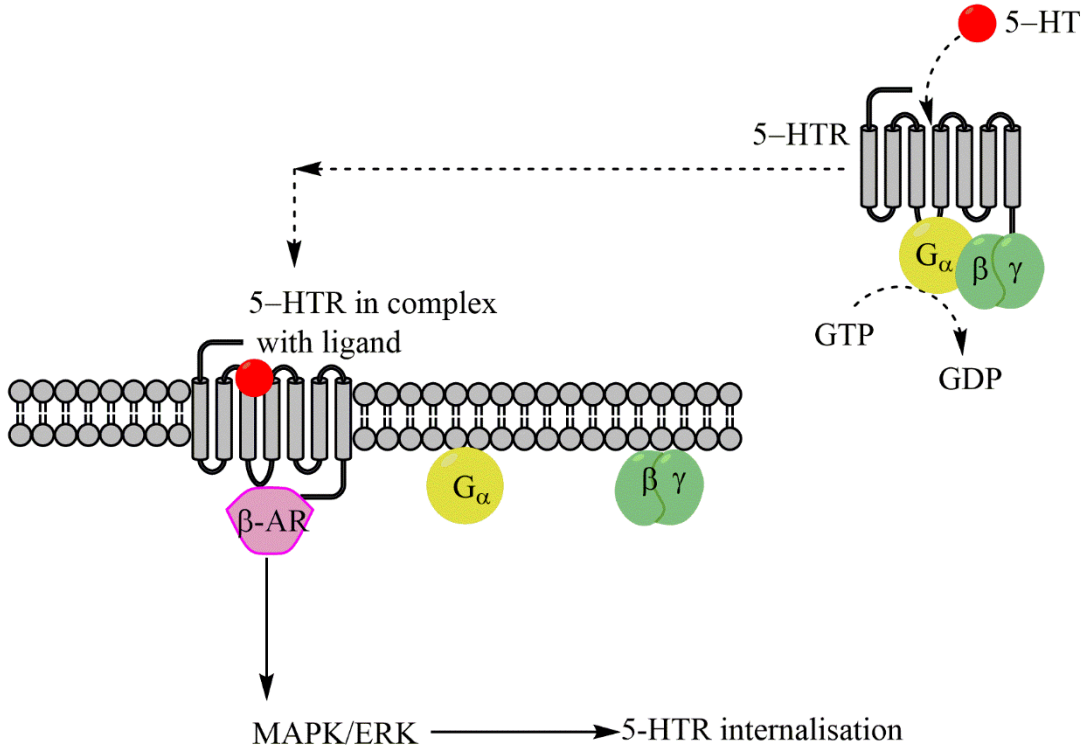


Figure 1.23 Depicting 5-HTR signalling mechanism via β-arrestin pathway. (Gα: alpha subunit of G protein, β/ϒ: beta/gamma subunit of G protein, β-AR: β-arrestin, ERK: extracellular signal–regulated kinases, MAPK: mitogen activated protein kinases, GTP: guanosine triphosphate, GDP: guanosine diphosphate)

Upon ligand activation of 5-HTR, a conformational change occurs at the cytoplasmic region of the receptor, which exposes G_α, G_β & G_γ subunits along with the bound GDP and they (G protein subunits) dissociate from 5-HTR upon exchange of GDP to GTP by G_α subunit. Upon further conformational change during the dissociation from G protein, more AA

residues from the receptor get exposed, which then undergoes phosphorylation via G protein-coupled receptor kinases (GRKs), which then recruits β-arrestin, leading to receptor internalisation process in a clathrin mediated fashion and the signalling can still be continued via MAPK activation during the receptor internalisation (52, 56, 57, 59, 69, 74, 75).

1.5.6 G protein mediated pathways

We still lack a complete understanding of the molecular downstream mechanisms of different 5-HTR subtypes, however based on the available information from various published articles, we can assume that there are several possible G protein mediated pathways (especially G_q & G_s mediated pathway have unique recruiting molecules), and different ligands induce conformational change in a unique way, which in turn leads to exposure of different AA residues at the cytoplasmic region of 5-HTR, and the subsequent signalling are tissue specific and effector protein specific. These cell signalling can bring about changes in the flow of ion channels as well as bringing changes at the DNA induced cellular level changes, which could be at both physiological level or functional level changes of the cell, which is depicted in Fig. 1.24 (59).

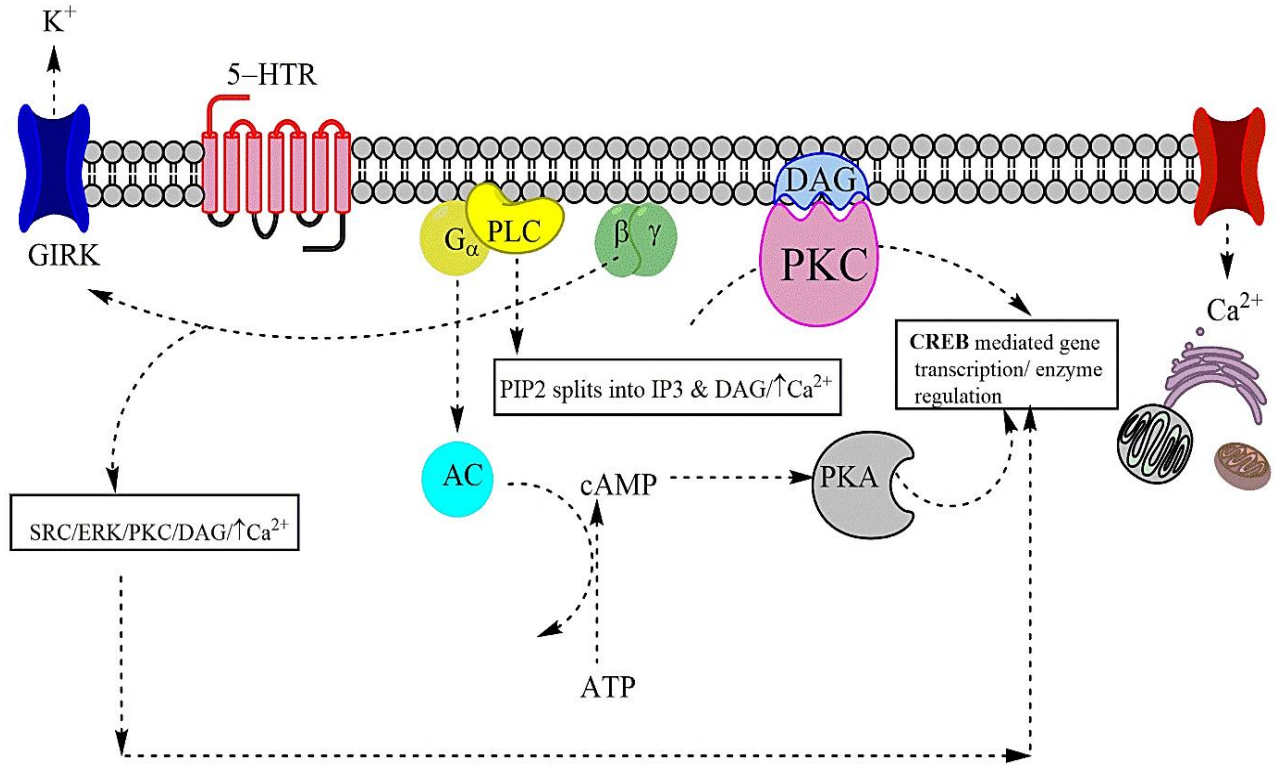


Figure 1.24 Serotonin receptor signalling mechanism via G protein mediated pathway. (GIRKs: G protein-coupled Inwardly-Rectifying potassium ion channels (hyperpolarises the cell when activated by βϒ subunits of G protein), PLC: Phospholipase C (upon activation by G_α subunit of G protein, PLC splits (via hydrolysis) PIP2 into IP3 and DAG), PIP2: Phosphatidylinositol 4,5-bisphosphate, IP3: Inositol 1,4,5-trisphosphate (enters cytoplasm and binds to IP3 receptors on smooth endoplasmic reticulum to release Ca^{2+}), DAG: diacylglycerol (activates PKC), PKC: Protein Kinase C (phosphorylates cytosolic proteins), AC: Adenylyl cyclase (converts ATP to cAMP), ATP: Adenosine Triphosphate, cAMP: Cyclic Adenosine Monophosphate (activates PKA), PKA: cAMP-dependent Protein Kinase A (upon activation catalytic component separates from its regulatory component and phosphorylates other proteins and regulates cellular processes including metabolism).

1.6 Aims and objectives of the research

5-HT has affinities for a wide range of 5-HTR subtypes, including 5-HT_{1B}. Anti-migraine drugs have the potential to mimic 5-HT at their receptors, i.e. at the 5-HTR due to molecular structural analogies. Though these drugs can act as 5-HT or DA mimics, their MOA as anti-migraine is still not clear, therefore, our aim of this research is also to throw some light in this area by finding out if these drugs can act as mimics and bind to 5-HT receptors and correct the irregularities in 5-HT or DA neurotransmission leading to relieving the symptoms in migraine.

1.6.1 Aims of the research

To study *insilico*, the structural analogy of anti-migraine drugs to 5-HT/DA, and their possible interactions at 5-HT/DA receptors due to their common functional groups and molecular structures.

1.6.2 Research objectives & study design

Using molecular modelling techniques - ChemBio3D, Pymol & Schrödinger;

I. Compare molecular structures of anti-migraine drugs (triptans & non triptans) with neurotransmitters such as 5- HT or DA and study the role of structural analogy between the drugs and neurotransmitters.
II. Explore the neurotransmitter's role in migraine using online search engines.
III. Download X-ray crystallographic coordinates of 5-HT/DA receptors
IV. Test docking of anti-migraine drugs as ligands to 5-HT/DA receptors and study the differences in interactions (in terms of binding energy, conformational changes, glide scores etc.) v/s their natural ligand.
V. Study, natural ligand-receptor crystal structures of 5-HT/DA receptors as references, possible interactions in their binding cleft & molecular conformations of 5-HT receptor complex.

VI. Validate the above in silico tests and Use appropriate references for the study design.

VII. Determine if anti-migraine drug treatment can be made more efficient by activating a 5-HT receptor and would a successful docking experiment can throw some light in the dark areas of understanding the migraine disorder.

VIII. Can this research lead to a better understanding of the migraine disorder and thus perhaps opening more possibilities for better treatment options such as structure-based drug design?

1.7 Research hypothesis in relation to the structural analogy concepts

- I propose through this research that a disturbance in the DA and 5-HT homeostasis is assumed as we don't have enough scientific evidence to derive this as a definite cause based on the findings as explained above, as I assume increased DA availability in the brain could be implicated in the development of Migraine, which could be aggravated by the phenomenon of molecular mimicry of these neurotransmitters to specific dietary AA (from chapter 1, Fig 1.1, also, please refer sec.1.4) along with other genetically predisposed factors, which have contributed to a lower threshold to migraine specific triggers, activates the migraine response and that anti-migraine drugs which mimic serotonin is partly able to correct this dyshomeostasis by activating certain 5-HTRs and able to relieve the symptoms.
- To simulate this molecular structural analogy, I have used *insilico* docking experiments with x-ray crystallographic structures of active 5-HTRs bound to ergotamine and anti-migraine drugs to dock in the ligand binding cleft (LBC) by replacing the bound ligand and studied the binding energies to see if they favour the interactions in a similar way within the permissible regulations of molecular binding dynamics. More details of this experiment can be found in the following chapter.

1.8 Methods

1. Prepare, a list of anti-migraine drugs commonly prescribed in migraine, and investigate the potential molecular mimicry between these drugs/or their metabolites (using published metabolism studies) and 5-HT/DA using online search tools such as University of Canterbury library online search tools, google scholar, google search, and online chemical information databases such as pubchem etc.
2. Use online data bases such as the protein data bank (PDB) (https://www.rcsb.org/) for appropriate protein receptors, and study the binding cleft using the natural ligand molecular structures and other published research documents to refer and verify interactions. Download X-ray crystallographic coordinates of 5-HT/DA receptors (include only important sub types, e.g., ERG-5-HT_{1B} receptor) and upload to Pymol/Schrödinger, locate their binding clefts with ligands and study the ligand – receptor binding mechanisms.
3. Attempt to dock anti-migraine drugs, in the binding cleft of 5-HT/DA receptors, using Schrödinger software. Determine binding mechanisms; use measurements and scoring functions such as glide factors and binding energy.
4. Study reference structures, if no bound natural ligand-receptor available, create one using methods from Schrödinger (remove ERG in ERG-5-HT 1B receptor complex, and dock natural ligand)

1.9 Overview of 'Glide' program and 'Maestro' interface from Schrödinger platform

Schrödinger provides a next generation web-based platform, with a diverse set of software tools designed for biochemical research and application. Maestro as an interface, organizes access to the interactive tools for use in materials science projects within Schrödinger (76).

Features of Maestro include various tool bar options, menu bar options, work space facilities, and multiple configuration tools (77).

1.9.1 Glide

Glide is a grid-based ligand docking software program with scientific methods and computational procedures. Glide can be run to create several ligand conformations, using ligand preparation protocol within the protein preparation wizard.
in a project designed by the user, one can generate several possible ligand conformations as poses of the ligand using ligand preparation protocol, where a rotatable group (Fig.1.25) will be identified automatically and saved as a new entry of ligand in a project and each entry can be used for docking experiment. Each ligand must be a single molecule, while the receptor may include more than one molecule, such as a protein. The ligand conformations (Fig.1.25) that Glide generates is docked with the receptor, using glide commands, after a series of receptor preparation procedures and the receptor-ligand interaction dynamics can be studied using the results output data obtained after running the protein preparation wizard protocol.

1.9.2 Glide: Protocols

Use appropriate work directory to save experiment results before starting the experiments. Following are the various stages of the experiments

1.9.2.1 Part A: Preparation of receptors

From the protein preparation wizard tab download the receptors using the PBD code (such as 4IAQ, 4IAR etc) and study the receptor with a bound ligand at the Ligand Binding Cleft (LBC) of the receptor.
Use Import and Process options from the software to prepare the receptor and trace any

- missing side chains
- missing loops

Use Review and Modify options to trace water molecules left in the protein after pre-processing, water molecules can be important in ligand binding especially when they are

near the LBC. On successful binding of the ligand, displacement of water molecule can be observed or sometimes they engage with the receptor or ligand to contribute to the stability of conformations via hydrogen bonding. Hence if water molecules are there in the LBC they may have a role sometimes in maintaining a receptor-ligand complex, hence no need to remove them. However, in distant side chains where you don't expect an interaction, they can be removed to save time in computational calculations.

H-bond assignment can be refined to minimize hydrogens of altered species (many missing sidechains will be detected to fill the valences appropriately) and optimized for their rotational options. Restrained energy minimization option is selected to aid minimum energy level calculations for scoring functions

1.9.2.2 Part B: Generation of the grid file

This part of the experiment defines the receptor grid where the appropriate ligands of choice will be docked.

By using receptor grid generation option, the receptor and ligand can be appropriately identified within the LBC, and the already bound ligand can be removed from the LBC.

A receptor grid box will be generated at this stage.

Rotatable groups within LBC can be identified and if required can be made rotatable, however the receptor backbone structure will be rigid in the whole experiment.

A grid file will be generated, and this will be used at a later stage of the experiment.

1.9.2.3 Part C: Ligand preparation

Use the 2D sketcher to draw the ligands for docking experiments.

The newly created ligand entry can be saved in the experiment project table with appropriate labelling.

The 2D structure can be converted to 3D in the workspace and can be explored to study the structural analogy. LigPrep tool can be used to prepare the ligand and generate several numbers of poses. Limiting the poses to a maximum of 20 would be enough to generate several low energy level conformations suitable for docking. This process will generate a file recording the ligand poses which will be used later during the docking process.

1.9.2.4 Part D: Ligand docking

Using the ligand docking tool, the receptor and the ligands prepared earlier will be used for docking experiment and an appropriate file will be generated. Several settings can be selected or removed such as tracking and using the appropriate files and using certain precision modes and output file descriptions etc. Several of the settings are appropriate to leave as default.

1.9.2.5 Part 5: Analysis of docking results

Using the XP visualiser option one can study the result table window and the various scoring functions. Select the ligands which have docked best by comparing the docking scores/glide scores. Examine the binding energies of each of the amino acid residues. One can look at the 3D interactions by opting for the several display options and study the structures and their interactions in the display workspace. Workspace gives a range of options such as pi-pi interaction and hydrogen bonds, as well as the good, bad and ugly interactions too. To look at 2D interactions simply click on the ligand interaction diagram tab which will highlight the residues and selected display interactions. The images can be saved to discuss in the research articles.

1.10 Ligand docking experiments using Schrödinger

- Limited number of flexibilities of rotatable groups

During the receptor grid generation and the ligand preparation stages there is a scope of allowing selective rotatable groups to be rotatable, which is not enough to mimic the real biological scenario, hence there will be some limitations to the reproducibility of experiment results in real biological systems. However, this experiment gives a picture of some reliable understanding of what we can expect in a ligand-receptor interaction with detailed binding energy data from several aspects.

There could be several conformations possible at the time of binding, which will depend on several electrostatic forces, however, some of this is captured in ligand, by generating 5 to 10 conformations per ligand and a few more at LBC of receptor residues (Fig.1.25).

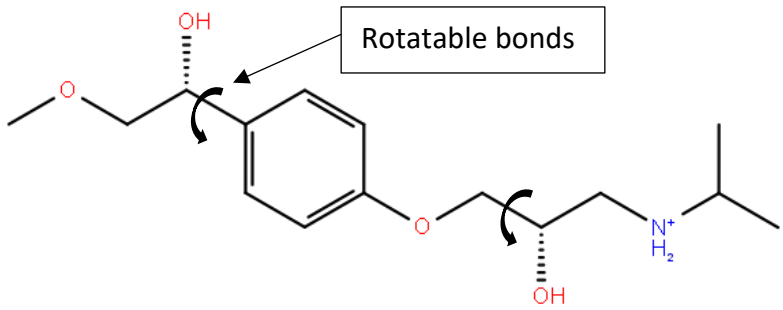


Figure 1.25 An example of rotatable groups in a molecule

As illustrated in Fig.1.25, each ligand is identified with a rotatable group by considering energy minimised poses by the software itself.

- Ligand selection criteria

Selected a list of anti-migraine drugs based on the current prescription trends, using online search options, published meta-analysis records, articles and journals.

Below is a list of ligands (Table 1.1) used in the docking experiment;

1. Active gabapentin	**2. Amitriptyline**
3. Aspirin	4. Dopamine
5. Droperidol	6. Eletriptan
7. Ergotamine	8. Gabapentin
9. Metoclopramide	10. Metoprolol
11. Nortriptyline	12. Propranolol
13. Serotonin	14. Sumatriptan
15. Topiramate	16. Verapamil
17. Metabolite of verapamil	18. Metabolite of amytriptyline
19. Metabolite of nortriptyline	20. Metabolite of norverapramil
21. Metabolite of aspirin	22. Metabolite of metaclopramide
23. Metabolite of metoprolol	24. Metabolite of propranolol
25. Paracetamol	26. Metabolite of paracetamol
27. Clozapine-antagonist	28. Cyproheptadine-antagonist
29. Cyproheptadine-antagonist	30. Loxapine-antagonist
31. Loxapine-antagonist	32. Risperidone-antagonist

Table 1.1 List of ligands used in the docking experiment.

- Receptor selection criteria

X-ray crystallised protein structures of 5-HTRs were used in this docking experiment. Below is a list (Table 1.2) of these receptor's ' PDB ID's along with their 5-HTR subtype classification;

1. 4IAQ-5HT1_B
2. 4IAR-5HT1_B
3. 4IB4-5HT2_B
4. 4NC3-5HT2_B
5. 5AIN-5HT3
6. 5TVN-5HT2_B
7. 5V54-5HT1_B
8. 6BQG-5HT2_C
9. 6BQH-5HT2_C
10. 6CM4-D_2
11. 5TUD-5HT2_B

Table 1.2 List of receptors used in the docking experiment

- Receptor and ligand preparation

Rigid receptor conformations bound to a ligand were used for the docking experiment where a ligand of choice with varying rigid conformations were used. These ligand

conformations were generated at the time of ligand preparation stage. Ligand docking were performed using Glide in extra precision (XP) mode. During receptor grid generation process, selected residues were identified by the program to allow rotation of hydroxyl and thiol groups.

Ligand preparation program were carried out with inbuilt following conditions;

I. All structures must be three-dimensional (3D).
II. They must have realistic bond lengths and bond angles.
III. No molecules with covalent bonds to the receptor.
IV. No accompanying fragments, such as counter ions and solvent molecules.
V. All atoms must be with filled valences of electrons and no missing hydrogens.
VI. Protonation state must meet the criteria appropriately considering pH values around 7 in real biological systems, for example, carboxylic acids should be deprotonated, and amino groups get protonated considering their pK_a values.
VII. A neutral aliphatic amine group can act as a hydrogen-bond acceptor without incurring any penalty.
VIII. Protonation is crucial when receptor site is a metalloprotein.
IX. All compatible formats including PDB is converted to Maestro format during any import processing, to facilitate Glide jobs.

- Receptor grid generation

In the receptor structure, an already bound ligand is selected to be identified as a ligand molecule within the ligand/receptor complex which is then distinguished from the receptor and removed after grid generation. The grid generated will appear as an enclosed box region of LBC, represents the volume of the protein for which grids will be calculated. Under *Van der Waals radii scaling* option, receptor atoms defined as nonpolar by a partial charge threshold during ordinary docking process is set by default values of scaling factor of 1.00, which means that the receptor atom radii are not changed, if this factor is changed then van der Waals radii of nonpolar receptor atoms are multiplied by this value. Some non-polar receptor atoms may attain partial charges during the docking process, and this can be made less significant as recommended during the energy scoring process by assigning a

slightly higher partial charge threshold value such as 0.25 to consider it to be non-polar, as *Van der Waals radii scaling* option is performed only on nonpolar atoms.

- Glide docking and results

Each ligand conformation is docked in the LBC, and scored in terms of its E-model, which comprised of significant weighting of the force fields and energies. Each Ligand, during conformation generation process described earlier, is represented by a set of core conformations, which depends on the number of rotatable bonds. For each core conformation, an exhaustive search of possible locations and orientations is performed at the LBC of the protein. Glide positions the ligand if there is a good enough match, and examins the placement of atoms that lie within a specified orientation of the ligand diameter, without many steric clashes with the receptor. The interactions of all atoms capable of making hydrogen bonds or ligand-metal interactions with the receptor are scored as subset test. The scoring depends on the ligand conformation and energy minimisation at recognised contacts within LBC. The scoring also recognizes favourable hydrophobic, hydrogen-bonding, & metal-ligation interactions, and penalizes steric clashes.

Finally, the selected poses are re-scored using Glide score based on Chem score, by adding other rewards and penalties devised by Schrödinger, for example, penalties for electrostatic mismatches known as buried polar terms, amide twist penalties, hydrophobic enclosure terms, and excluded volume penalties etc.

An example of Glide score (G score) calculation for a ligand entry (conformation specific) is as shown below (77);
Glide score = 0.05*vdW + 0.15*Coul + Lipo + Hbond + Metal + Rewards + RotB + Site

(vdW: Van der Waals energy; Coul: Coulomb energy; Lipo: Lipophilic;
HBond: Hydrogen-bonding; Metal: Metal-binding; Rewards: Rewards and penalties; RotB: Penalty for freezing rotatable bonds; Site: Polar interactions in the active site)

The electrostatic forces and binding energies considered for scoring purposes were

comprised of;

1. Van der Waals energy: calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates etc.
2. Coulomb energy: calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates etc.
3. Lipophilic interactions: rewards favourable hydrophobic interactions.
4. Hydrogen-bonding: depend on whether the donor and acceptor are neutral, one is neutral and the other is charged, or both are charged.
5. Metal-binding: interactions with anionic or highly polar acceptor atoms are included. If the net metal charge in the apo protein is positive, the preference for anionic or polar ligands is included and if the net charge is zero, the preference is suppressed.
6. Rewards and penalties: such as buried polar groups, hydrophobic enclosure, correlated hydrogen bonds, amide twists, and so on.
7. Penalty for freezing rotatable bonds.
8. Polar interactions: polar but non-hydrogen-bonding atoms in a hydrophobic region are rewarded.

- Analysing ligand interactions using the docking results

Various scoring parameters available from the glide docking experiments for ligand interaction studies are;

1. Emodel: combines the Glide score and the nonbonded interaction energies. Generally used to rank each ligand entry within a project designed by the user. gives an indication of best-docked structure for each ligand. The score combines the energy grid score, the binding affinity predicted by Glide score, and in the case of flexible docking, gives the internal strain energy specific to conformational-search algorithm.

2. Coulomb-van der Waals interaction-energy score (Glide energy): formulated to avoid overly rewarding charge-charge interactions. This score is intended to be more suitable for comparing the binding affinities of different ligands.
3. Docking score: is the Glide score supplemented by Epik state penalties (state penalty for ligand protonation and tautomerization) and strain corrections in the case of induced fit docking
4. Glide score: as an estimate of the binding energy including hydrogen bonds, hydrophobic enclosure reward contributions and factors rewarding, or penalizing interactions known to influence ligand binding.
5. Ligand Interaction Diagram: a schematic representation of hydrogen bonds and π-π interactions.

Glide can reduce the time and cost involved in finding the best ligand search where different rotamer groups to be optimized one at a time for a given core conformation and location of the ligand. Glide offers performance advantages which allows large libraries to be screened at an affordable computational cost. The primary purpose of Glide program is for screening large numbers of ligands bound to a largely rigid receptor models (77). Glidescore (Gscore) is an empirical scoring function designed to maximize separation of compounds with strong binding affinity from those with little or no binding ability. As an empirical scoring function, it is comprised of terms that account for the physics of the binding process including a lipophilic-lipophilic term, hydrogen bond terms, a rotatable bond penalty, and contributions from protein-ligand coulomb-vdW energies.

Chapter 2 Results

2 Experiment results

The *insilico* modelling studies were carried out with Glide software program using Maestro interface from Schrödinger (Schrödinger Release 2017-1: Maestro, Schrödinger LLC, New York, NY, 2017 & Schrödinger Release 2018-3**:** Glide, Schrödinger, LLC, New York, NY, 2018). Glide allows several options for the ligand docking experiments such as, specifying the receptor grid for docking, making ligand settings for glide docking, making basic settings for glide docking, constraining ligand docking to a specified core etc. Some of the above options were opted and further specified while running the program as mentioned below;

- The Glide mode opted for ligand docking were of extra precision (XP) mode, which helps in visualising some of the ligand interactions.
- Van der Waals radii scaling for ligand atoms was set to the default values with scaling factor to 0.8 and partial charge cut off to 0.15 (to soften the scoring of the for non-polar parts of ligands).
- Opted for flexible ligand sampling, as this gives better docking chances over rigid ligand.
- Opted to add epic state penalties to docking score, where epik uses empirical Hammett and Taft relations to predict pK_a values by recognizing functional groups that may be ionized by the addition or removal of a proton based on the pH values of the solution.
- Ligand core restricted to reference position of the ligand already bound in receptor.
- Restricted ligand interaction scoring within 12 Å of grid centre.
- Rigid receptor conformations were used with some flexibility allowed only to selected rotatable groups.

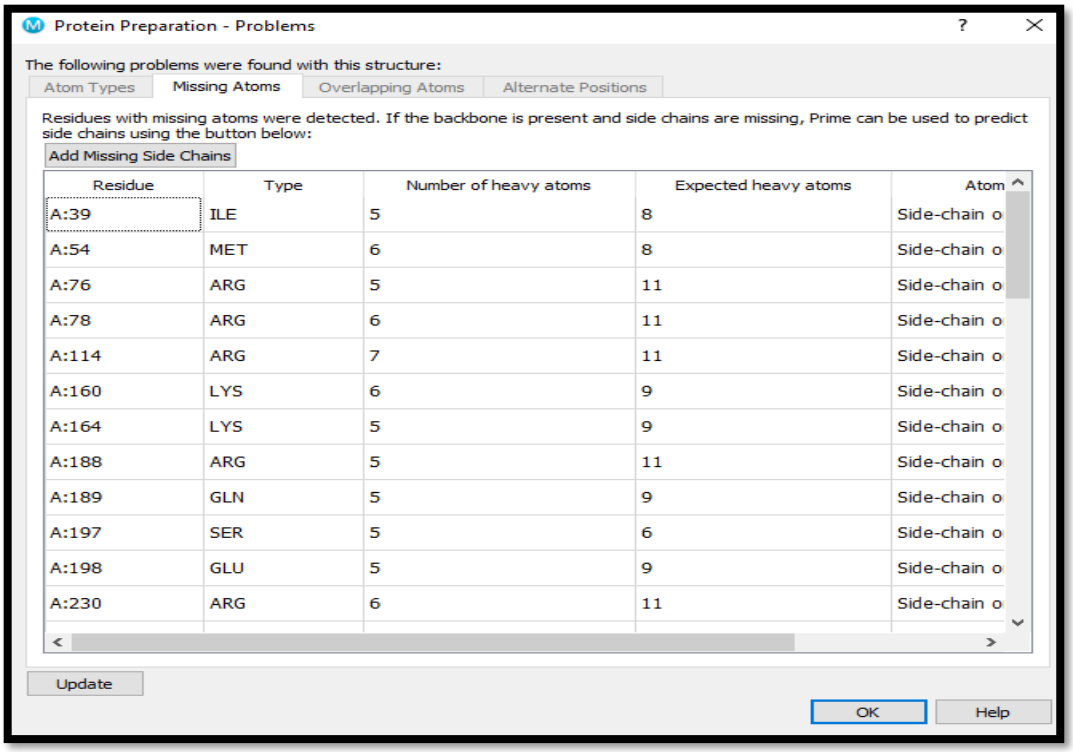
The *insilico* experiments were carried out using 32 molecules, most of them known to be prescribed as anti-migraine drugs, by general practitioners (GPs) for the treatment of migraine. These molecules were docked as ligands to a set of 10 known 5-HTRs and a single DA receptor subtype known as D_2. X-ray crystallographic structures of protein receptors such as proteins with PDB ID; 4IAQ, 4IAR etc., were downloaded from online protein data

bank. These protein receptor structures had at least one ligand bound to them, when they were crystallised, and this ligand-receptor complex and their interactions were identified as reference LBC and the bound ligand was identified as reference ligand during the *insilico* docking study. The 32 ligand structures, used for docking study, were built using 2D sketcher option from Maestro and were entered to a new project. Both ligands and receptors were prepared using LigPrep and Protein Preparation Wizard options respectively from Glide. After identifying the LBC as explained earlier, a receptor grid was generated using the Receptor Grid Generation option from Glide. This receptor grid uses already bound ligand as a reference ligand core for docking the other 32 ligands of our choice, by replacing the reference ligand in the protein receptor within 0.1 to 1 Å tolerance.

Glide program is generally used for screening applications for choosing preferred ligands from a pool of database of ligands. However, Glide can also be optimized for docking accuracies, which introduces some sensitivity to the receptor conformations studied for docking due to lack of receptor flexibility and is reflected in results as glide score (gscore). These ligands for the docking studies were built and prepared using LigPrep option from Glide, where a maximum of 20 ligand poses were generated. Some more details of ligand preparation and docking process are detailed in following sections.

2.1 Protein and ligand preparation results

At the time of receptor preparation, several residues were identified (Fig. 2.1) to fill with missing side chains (approximately 48 residues for 4IAQ), and during receptor grid generation several hydroxyl groups (or thiols) or hydrogen atoms at the LBC were made rotatable to facilitate ligand interaction (Fig. 2.2). Some examples of residues of $5HT_{1B}$ receptor subtype with rotatable groups were; Tyr40, Tyr109, Ser127, Cys133, Thr134, Tyr208, Ser212, Ser334, Thr355, Tyr359 etc. and examples of residues with filled in missing side chains of $5HT_{1B}$ receptor subtype were; Ile39, Met54, Arg76, Arg78, Arg114, Lys160, Lys164, Arg188, Gln189, Ser197, Glu198 etc.



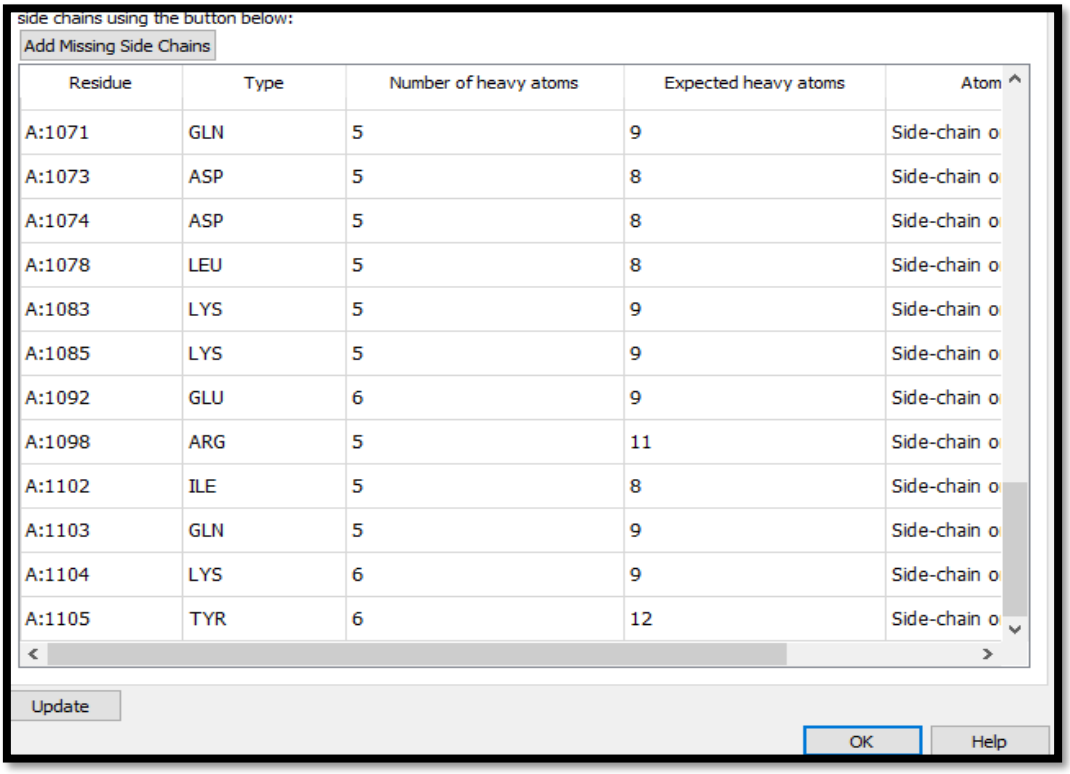
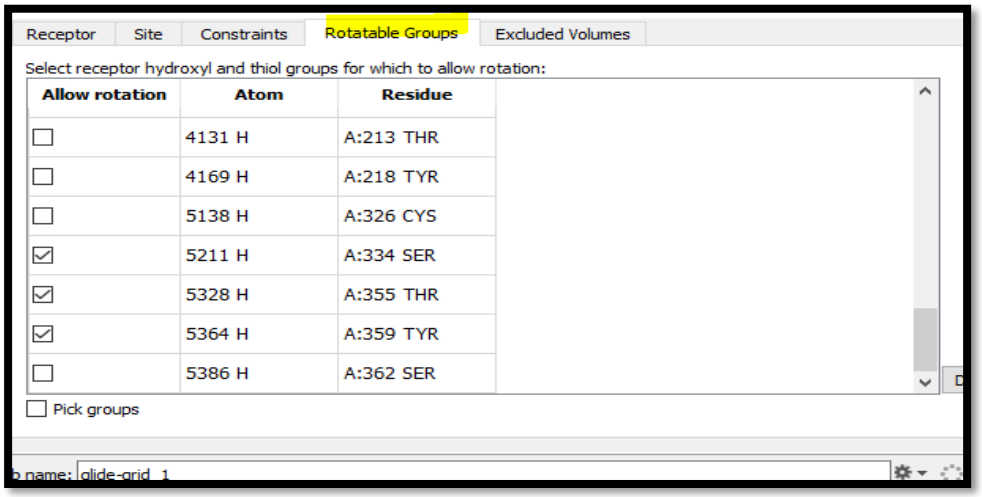
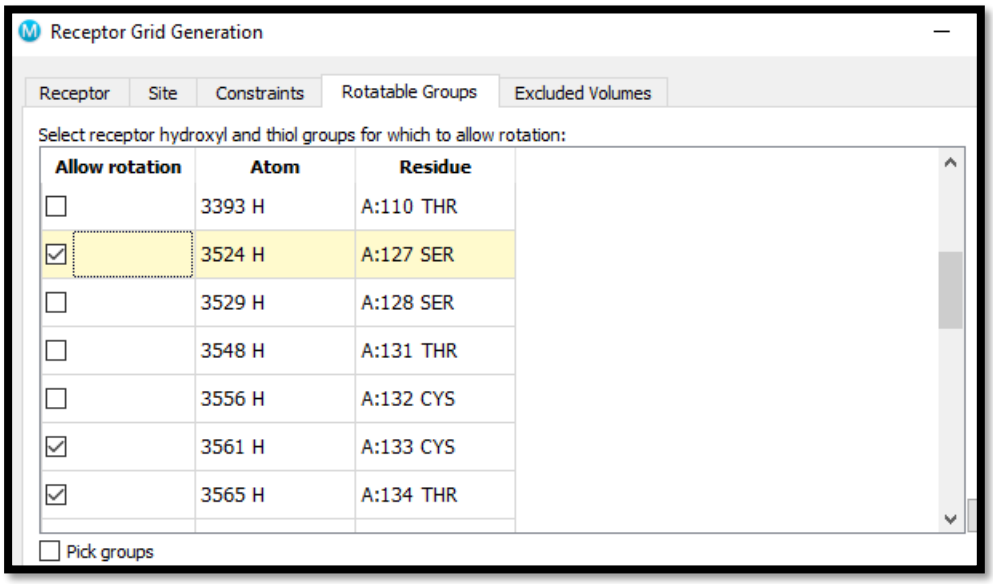


Figure 2.1 Above examples of Maestro window highlighting missing atoms detected by protein preparation wizard from Glide program for receptor PDB ID:4IAQ. The above displayed residues are examples of how we can locate different helices (Helix I/II/III) based on the residue number informations and study for their proximity to ligand for missing atoms of hydroxyl group or thiol groups.





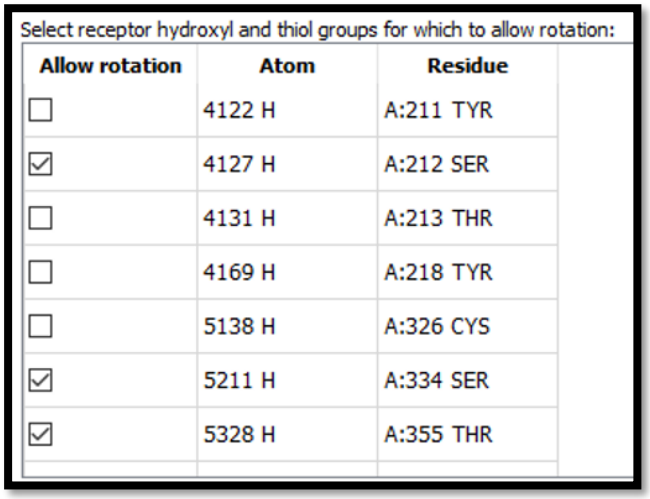


Figure 2.2 Examples of rotatable groups selected for receptor PDB ID: 4IAQ from the Maestro window, based on its proximity from the helices identified (based on the residue numbering) to the ligand where an addition of hydrogen atom may be crucial in establishing a ligand interaction such as hydrogen bonding.

2.2 Ligand docking results: G scores, docking scores & glide energy

When Ligand Docking was set up from Glide, following options were selected (Fig. 2.3);

- Precision set to XP mode
- Ligand sampling set to flexible
- Add Epik penalties to docking score

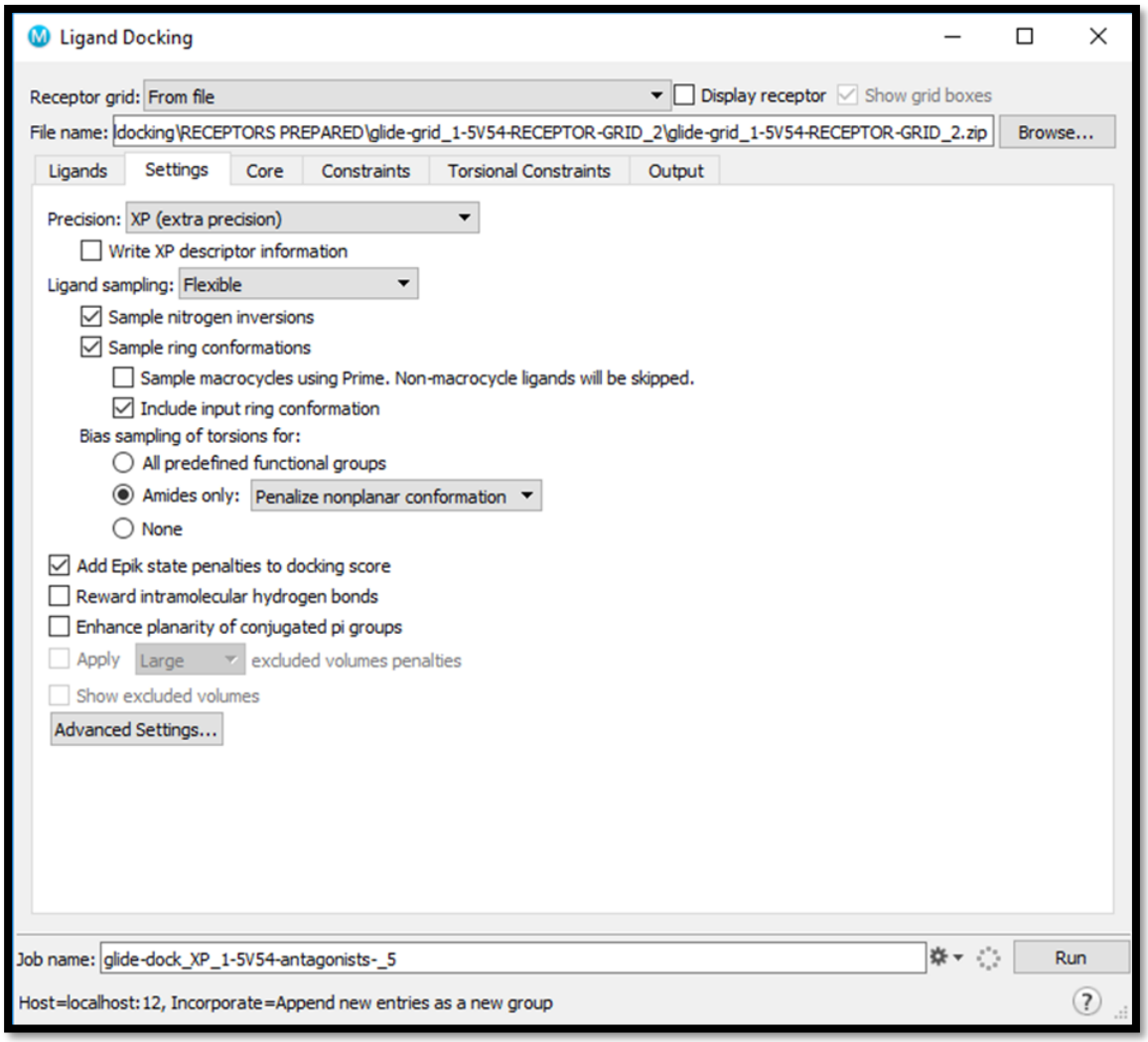
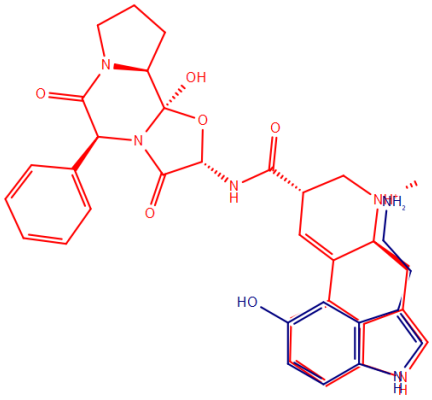
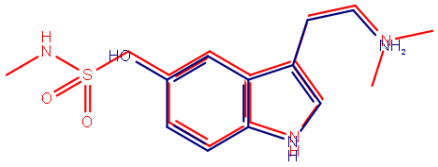


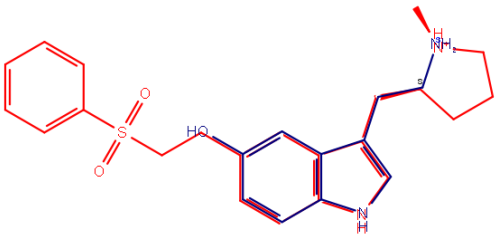
Figure 2.3 settings of ligand docking experiments from the Maestro window using Glide XP mode precision selection and the selections for penalising the non-appropriate conformations and scoring parameters with the option of flexible ligand.



Serotonin (blue) overlaid to Ergotamine (red)



Serotonin (blue) overlaid to Sumatriptan (red)



Serotonin (blue) overlaid to Eletriptan (red)

Figure 2.4 Striking structural analogy of serotonin (in blue) to anti-migraine drugs ergotamine, sumatriptan and eletriptan when overlaid.

When anti-migraine drugs were overlaid to serotonin or dopamine, most of them showed a visibly hidden serotonin or dopamine in their molecular structures (Fig. 2.4). Ergotamine bound 5-HTRs downloaded from protein data bank (PDB ID: 4IAQ & 4IAR), probably indicate that ergotamine may be mimicking 5-HT in its receptors and can activate 5-HTRs due to its structural analogy to 5-HT, especially due to an indole ring present in them. This could be one of the reasons of its analgesic property when ergotamines are prescribed in migraine. Hence, we can propose that, the same could be true with most of the anti-migraine drugs, and that they are either mimicking 5-HT or DA at their respective receptors and support the same assumption with the docking results of anti-migraine drugs to 5-HT & DA receptors.

Hence, a molecular modelling study was carried out to explore the possibility of docking antimigraine drugs to 5-HTRs/D_2 and tested if they can establish any desirable interactions to activate the receptors by recording their gscores in kcal/mol (Fig. 2.6). Interestingly all

anti-migraine drugs tested had negative energy values (in Kcal/mol) for all the three different parameters such as gscore, docking score and glide energy (Table 2.1).

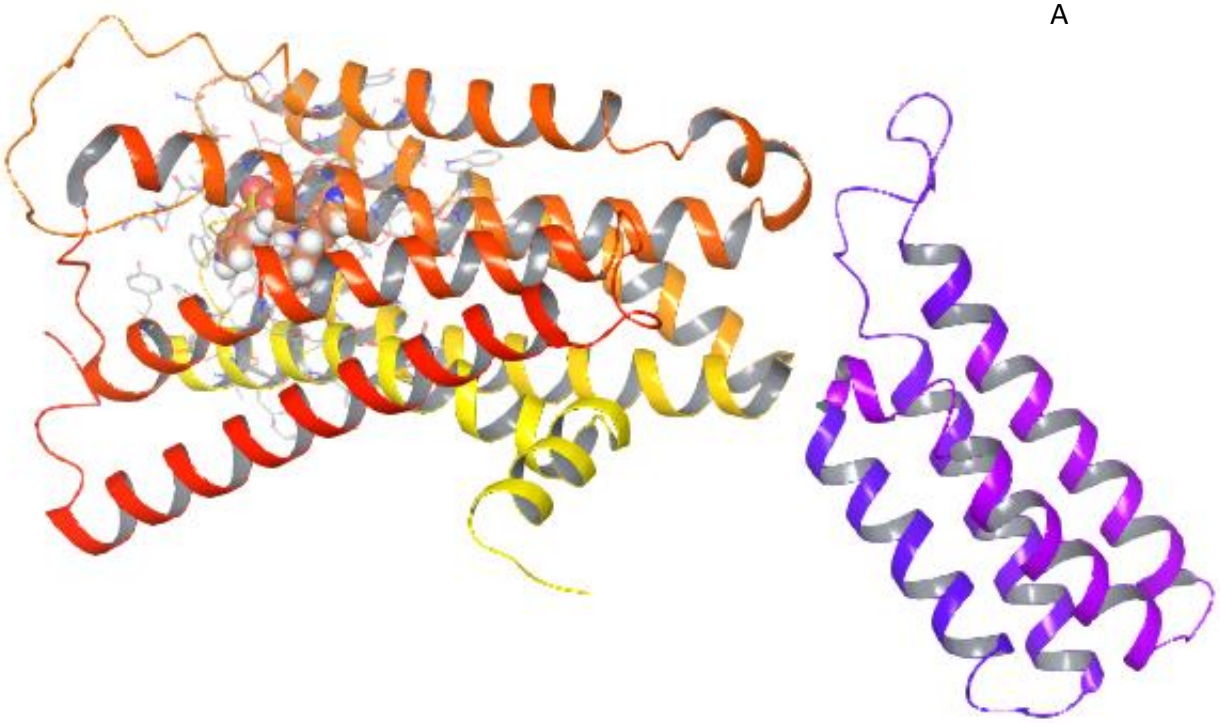
Title	Gscore	Docking score	Glide energy
Serotonin	-7.293	-7.293	-33.241
Eletriptan	-11.879	-11.877	-54.32
Eletriptan	-10.617	-10.615	-54.557
Sumatriptan	-10.079	-10.075	-49.693
Ergotamine	-10.295	-9.558	-62.744
Amitriptyline	-9.067	-9.064	-38.393
Nortriptyline	-9.007	-9.007	-33.466
Ergotamine	-9.021	-8.809	-64.636
Ergotamine	-9.432	-8.696	-55.856
Droperidol	-9.788	-8.676	-44.777
Serotonin	-8.2	-8.2	-32.824
Dopamine	-8.18	-8.18	-31.813
Droperidol	-8.192	-8.093	-46.966
Propranolol	-8.579	-8.065	-36.795
(Metabolite) Nortriptyline	-7.686	-7.686	-35.721
(Metabolite-EHNT) Amitriptyline	-7.546	-7.546	-36.941
Verapamil	-7.506	-7.505	-33.675
(Metabolite) Norverapamil	-7.452	-7.452	-53.056
Metoprolol	-7.364	-7.361	-40.044
Metoclopramide	-7.128	-7.123	-40.373
(Metabolite) propranolol	-6.838	-6.838	-39.129
Droperidol	-7.843	-6.731	-48.825
(Metabolite-D617) verapamil	-6.282	-6.282	-35.216
Propranolol	-6.498	-6.175	-37.733
(Metabolite) Salicycluric acid	-5.863	-5.863	-35.468
(Metabolite) Metoprolol	-5.78	-5.78	-43.011
(Metabolite) Metoclopramide	-5.727	-5.727	-36.818
Gabapentin	-5.674	-5.674	-14.042
Topiramate	-4.867	-4.867	-32.388
Active Gabapentin	-4.745	-4.745	-20.799
paracetamol	-4.034	-4.033	-27.746
Paracetamol (Metabolite-NAPB)	-5.24	-5.24	-30.089
Aspirin	-3.503	-3.503	-20.571

Table 2.1 Glide scores, Docking scores and Glide energies (units in Kcal/mol) for different molecules and their metabolites obtained after the *insilico* experiments of ligand docking to 5-HT1B (PDB ID 4IAQ) with Glide software from Schrödinger (Schrödinger Release 2017-1: Maestro, Schrödinger LLC, New York, NY, 2017 & Schrödinger Release 2018-3: Glide, Schrödinger, LLC, New York, NY, 2018).

All anti-migraine drugs used in the *insilico* docking study had a negative gscore to 5-HTRs, and lower the Gscore, would mean more successful the binding to the receptors studied. Most of the anti-migraine drugs tested including eletriptan, ergotamine, and sumatriptan had gscores lower than the natural ligand 5-HT, indicating a better binding possibility to 5HTRs (Fig. 2.7). Of all the ligands tested glide energies were lowest for Eletriptan and Ergotamine (Fig. 2.7 & 2.6), and docking scores were also lowest for these two along with sumatriptan (Table 2.1). However, at this stage we can't predict how likely they behave in real biological systems.

It is well addressed in the section 1.5.4 that 5-HT can regulate a wide range of signalling mechanism through 5-HT receptors and that their role in alleviating pain and inflammation in cephalic and peripheral nervous system and vascular regions is an interesting area to explore. When we explore the signalling mechanisms it is by now known that several residues which are crucial in recognising the conformational changes in the orthosteric binding pocket which spans around the transmembrane region to the extra cellular half of the receptor can act as micro switches to initiate a signalling mechanism. These signalling mechanisms recruit signalling proteins at their cytoplasmic region of the receptor as explained in section 1.5.4 to 1.5.6, initiating a cascade of signalling pathway. 5-HT or other look alike molecules such as anti-migraine drugs may initiate these signalling mechanisms by interacting with certain residues at their extra cellular area of the receptor (78). Certain residues within the receptor are key to recognising the ligand by acting as microswitches are capable of initiating conformational changes and can extend these conformational changes by displacing the helices in the cytoplasmic region (Fig. 2.9 & 2.10), which exposes regions for coupling with secondary signalling proteins. Some of these microswitches are known as PIF, DERY, NPY motiffs. The DERY motiff comprises of residues from 5-HT_{1B} are, Asp146, Glu309, Arg147, Tyr157 and from 5-HT_{2B} are, Asp152, Glu319, Arg153, Thr89 (78). Further investigations in this regard will be discussed under ligand interactions section in this chapter, where a detailed ligand-receptor complexes post docking experiments in Maestro will be shared.

A



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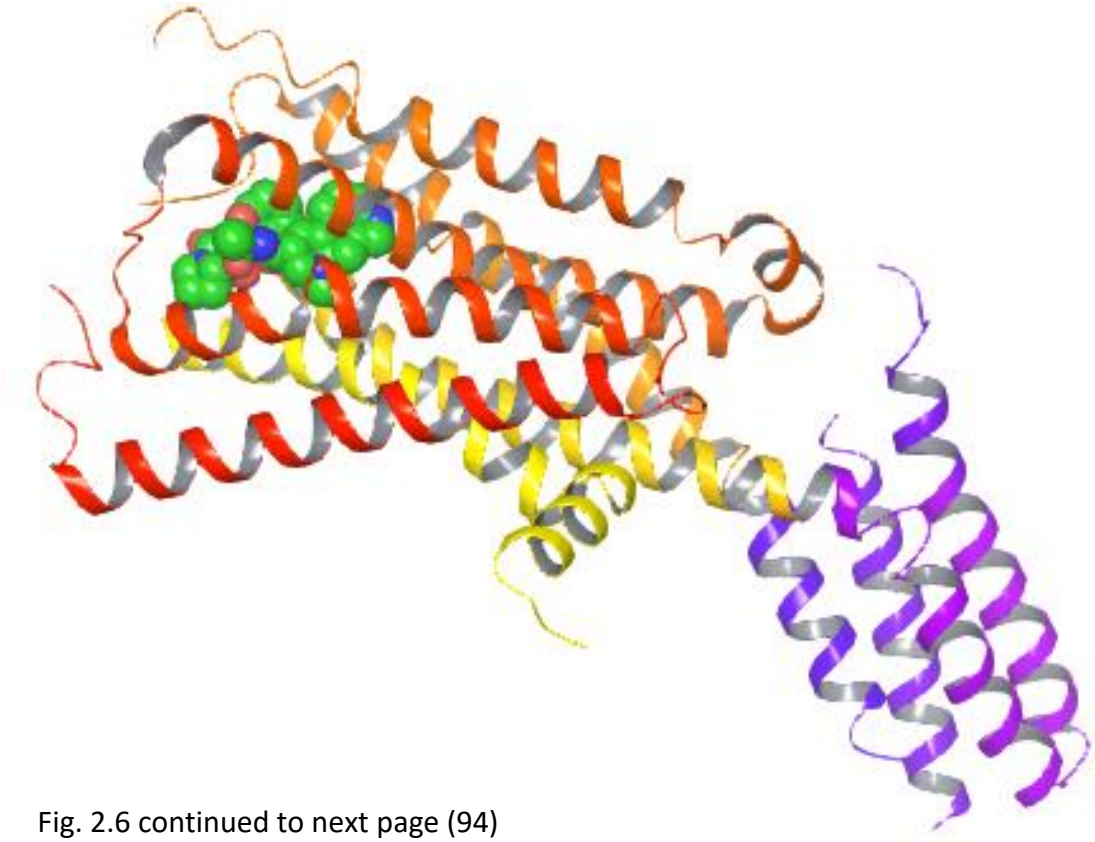

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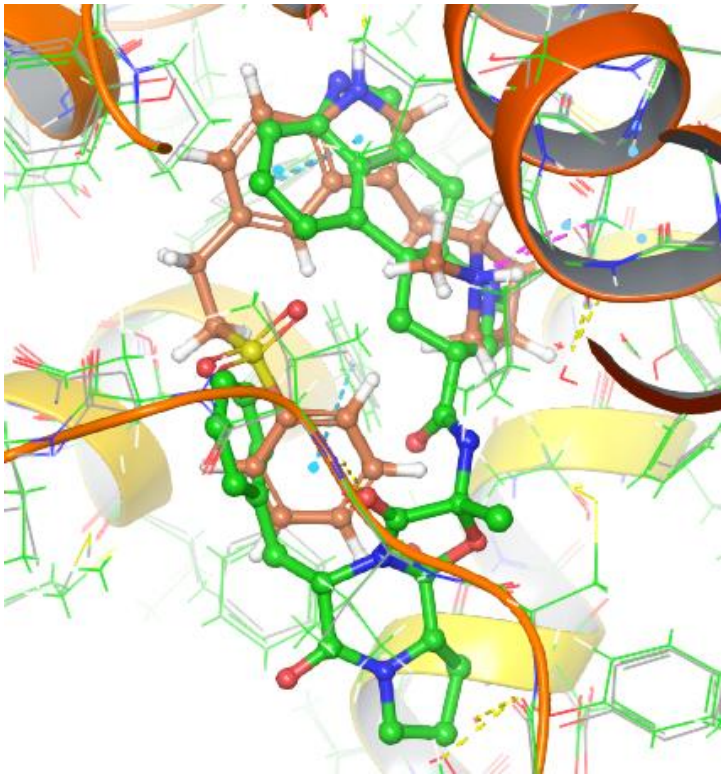

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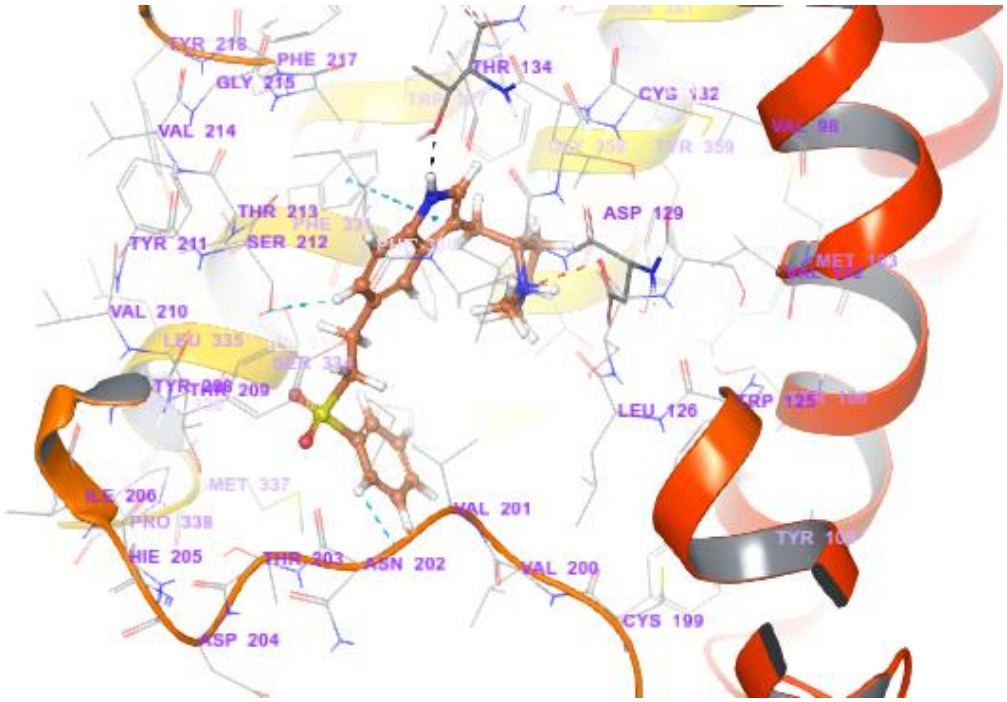

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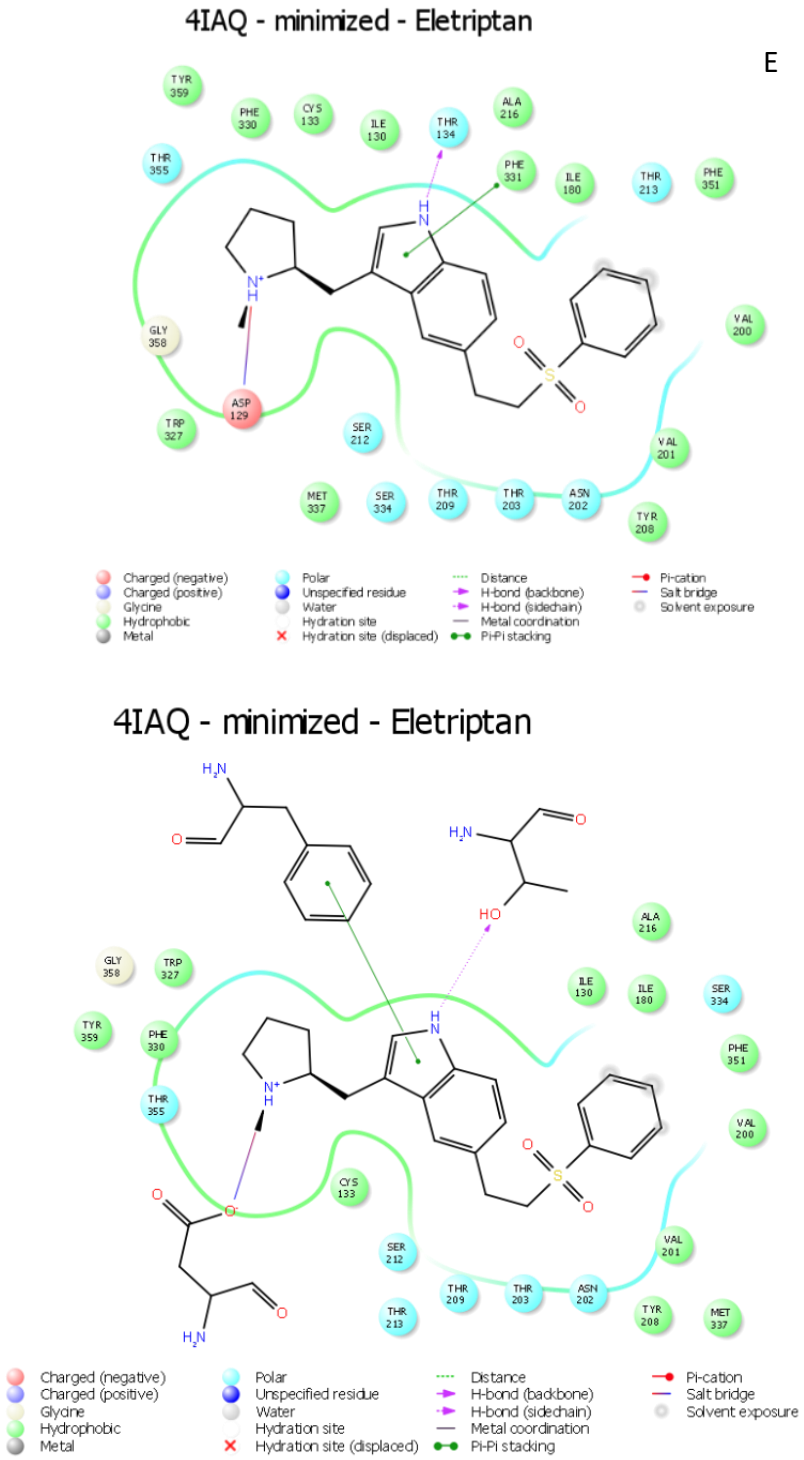
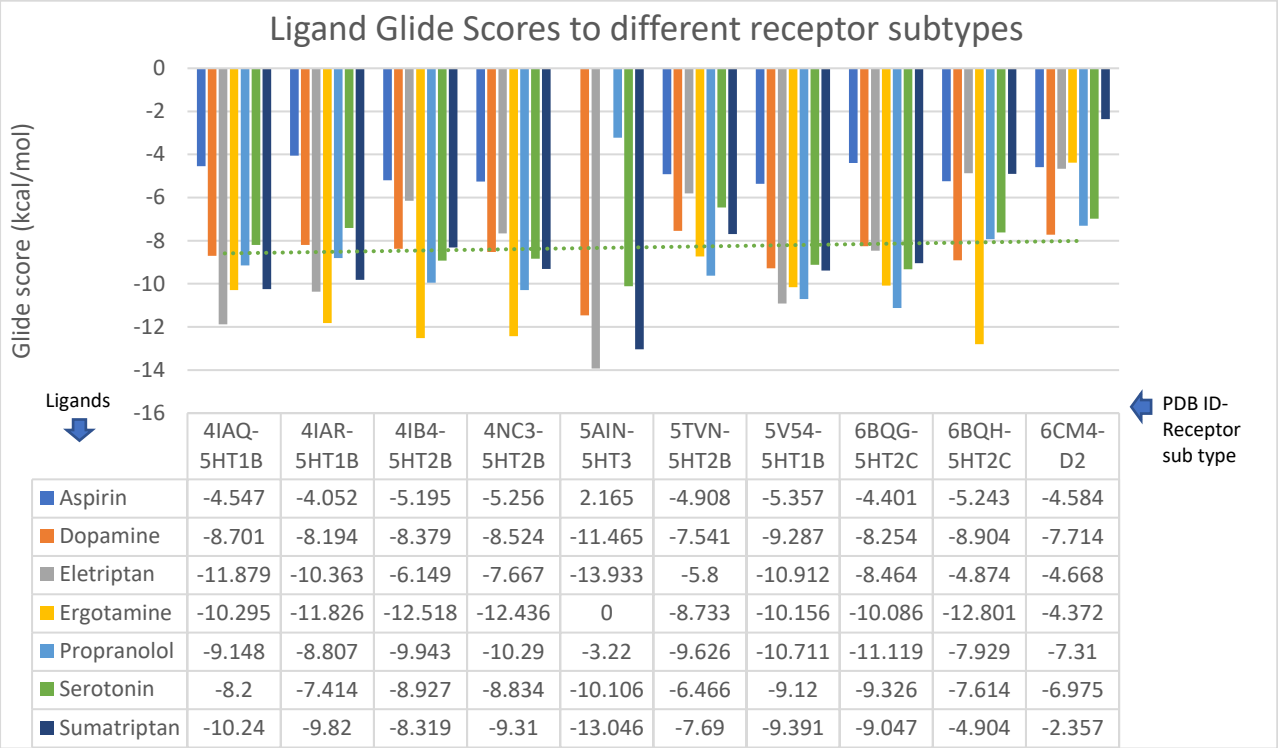


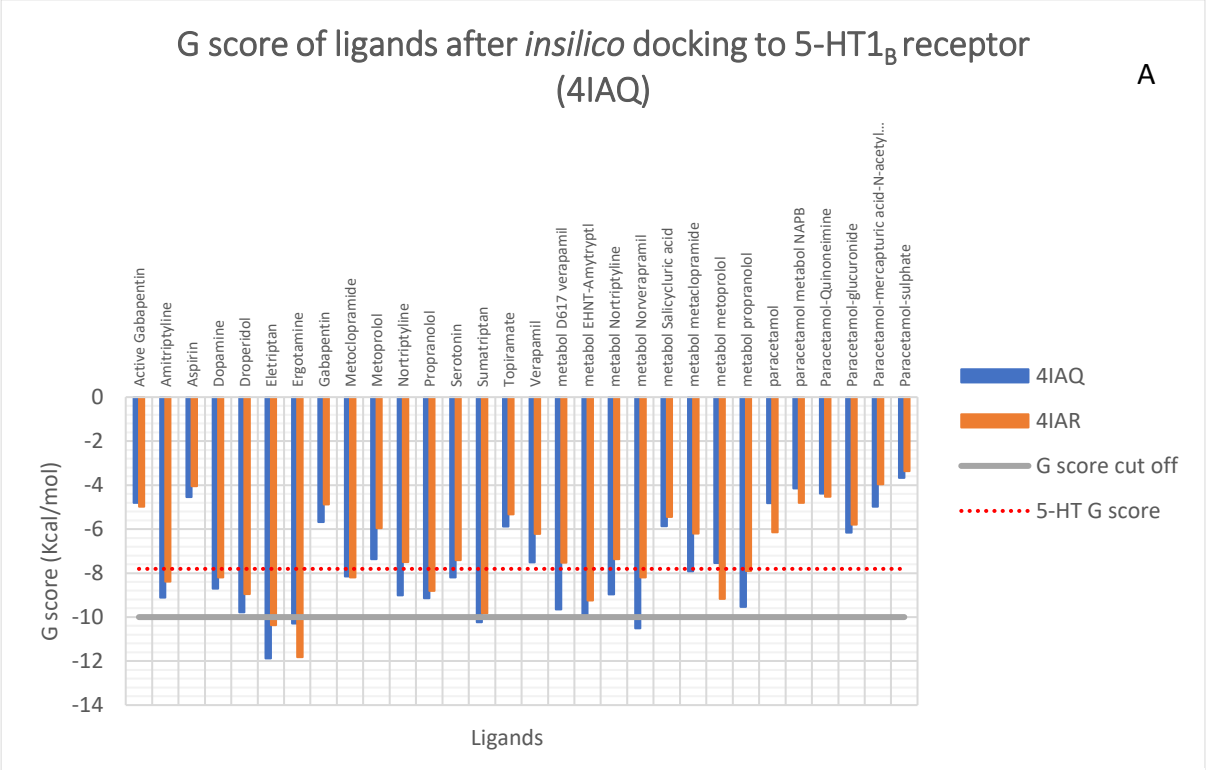
Figure 2.5 A) PDB ID: 4IAQ with bound Ergotamine (ERG) before docking experiment B) Eletriptan (ELE) post docking experiment to the same binding pocket C) Superimposed image of both ERG (in green) and ELE describing the conformational differences between both the ligands, and confirming docking at the same binding site D) ELE at the orthosteric binding pocket of 4IAQ, displaying salt bridge interaction (distance 2.8 Å) to ASP 129, H-bond (distance 1.9 Å) with THR 134 and a Pi-Pi stacking (distance 5 Å) with PHE 331, also detailed ligand interaction diagram in E & F. (5-HTRs used above are from online protein data bank with PDB ID: 4IAQ,and processed with Maestro from Schrödinger software platform)



Ligands	4IAQ-5HT1B	4IAR-5HT1B	4IB4-5HT2B	4NC3-5HT2B	5AIN-5HT3	5TVN-5HT2B	5V54-5HT1B	6BQG-5HT2C	6BQH-5HT2C	6CM4-D2
Aspirin	-4.547	-4.052	-5.195	-5.256	2.165	-4.908	-5.357	-4.401	-5.243	-4.584
Dopamine	-8.701	-8.194	-8.379	-8.524	-11.465	-7.541	-9.287	-8.254	-8.904	-7.714
Eletriptan	-11.879	-10.363	-6.149	-7.667	-13.933	-5.8	-10.912	-8.464	-4.874	-4.668
Ergotamine	-10.295	-11.826	-12.518	-12.436	0	-8.733	-10.156	-10.086	-12.801	-4.372
Propranolol	-9.148	-8.807	-9.943	-10.29	-3.22	-9.626	-10.711	-11.119	-7.929	-7.31
Serotonin	-8.2	-7.414	-8.927	-8.834	-10.106	-6.466	-9.12	-9.326	-7.614	-6.975
Sumatriptan	-10.24	-9.82	-8.319	-9.31	-13.046	-7.69	-9.391	-9.047	-4.904	-2.357

Figure 2.6 *Insilico* docking studies with Glide software from Schrödinger shows high potential for antimigraine drugs (when docked as ligands) to mimick 5-HT and be able to interact at binding sites of different 5-HT receptor subtypes especially for those with a G score (in kcal/mol) lower than that of 5-HT (as green trend line in above graph) itself, and one such molecule is Ergotamine (g score -11.8). Please note: as per Schrödinger g scores of -10 or lower usually represent good binding. However for hydrophobic interactions, g scores of -8 or -9 might be very good, and Glide XP tends to produce lower g scores (-12 or below),which depends on the van der Waals radii scaling factors (79, 80).

Several repeats of the docking experiments were performed, where results were similar for similar ligand conformations. However, some repeats were set to perform with increased number of different ligand conformations than previosly set, along with a few more increased number of rotatable residues near the binding site, while generating receptor grid, which resulted in improved docking scores and gscores. Out of all the repeat results, the highest negative glide scores were selected for result analysis table (Table 2.1) as the intention was to get the data, indicating any possibility of establishing ligand interactions with the studied receptors and anti-migraine drugs.



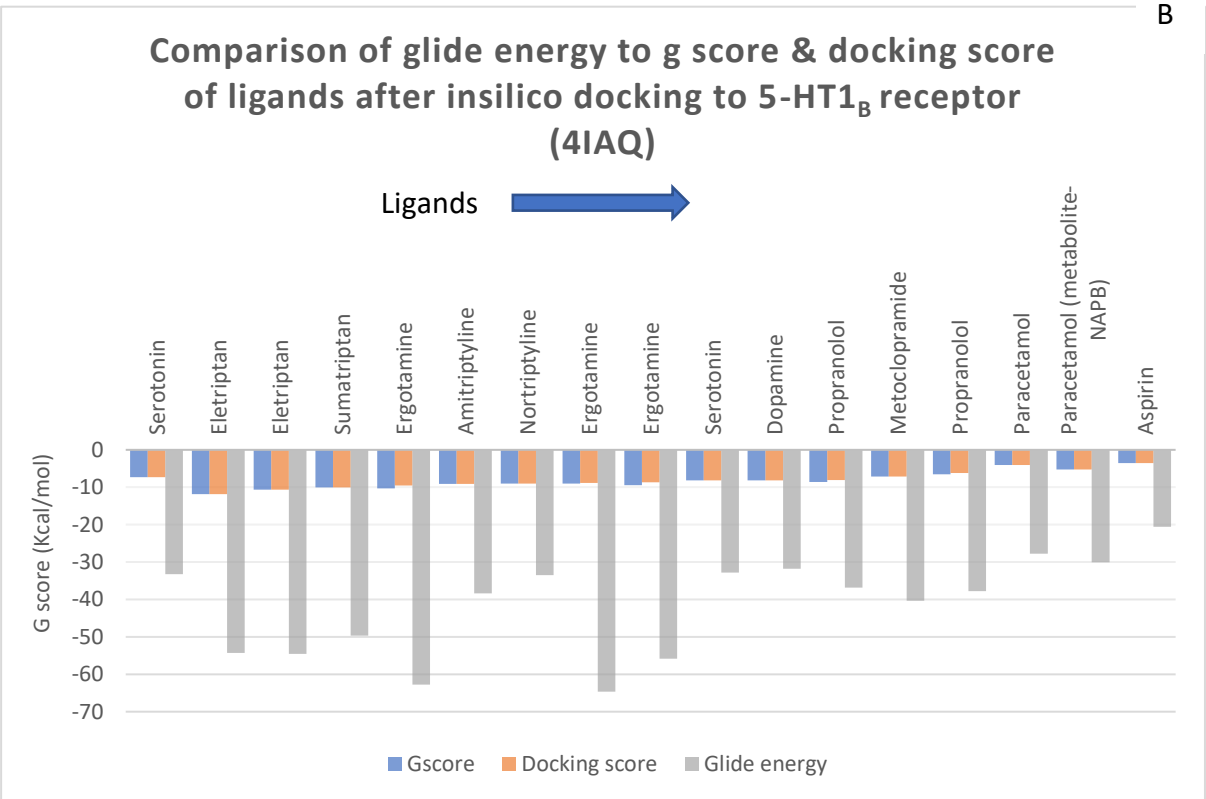


Figure 2.7 A) Glide scores (G scores) for a range of selected molecules, post *insilico* docking to 5HT1$_B$ receptors. G score of natural ligand, 5-HT and G score cut off value of -10 Kcal/mol are plotted as trend lines for comparison with other ligands. G score higher than or equal to 5-HT indicates a good binding, such as eletriptan and ergotamine. B) Comparison of Glide energy to both G score and Docking score shows penalty scoring has impacted in their low scoring. G score and docking score combines some additional penalty

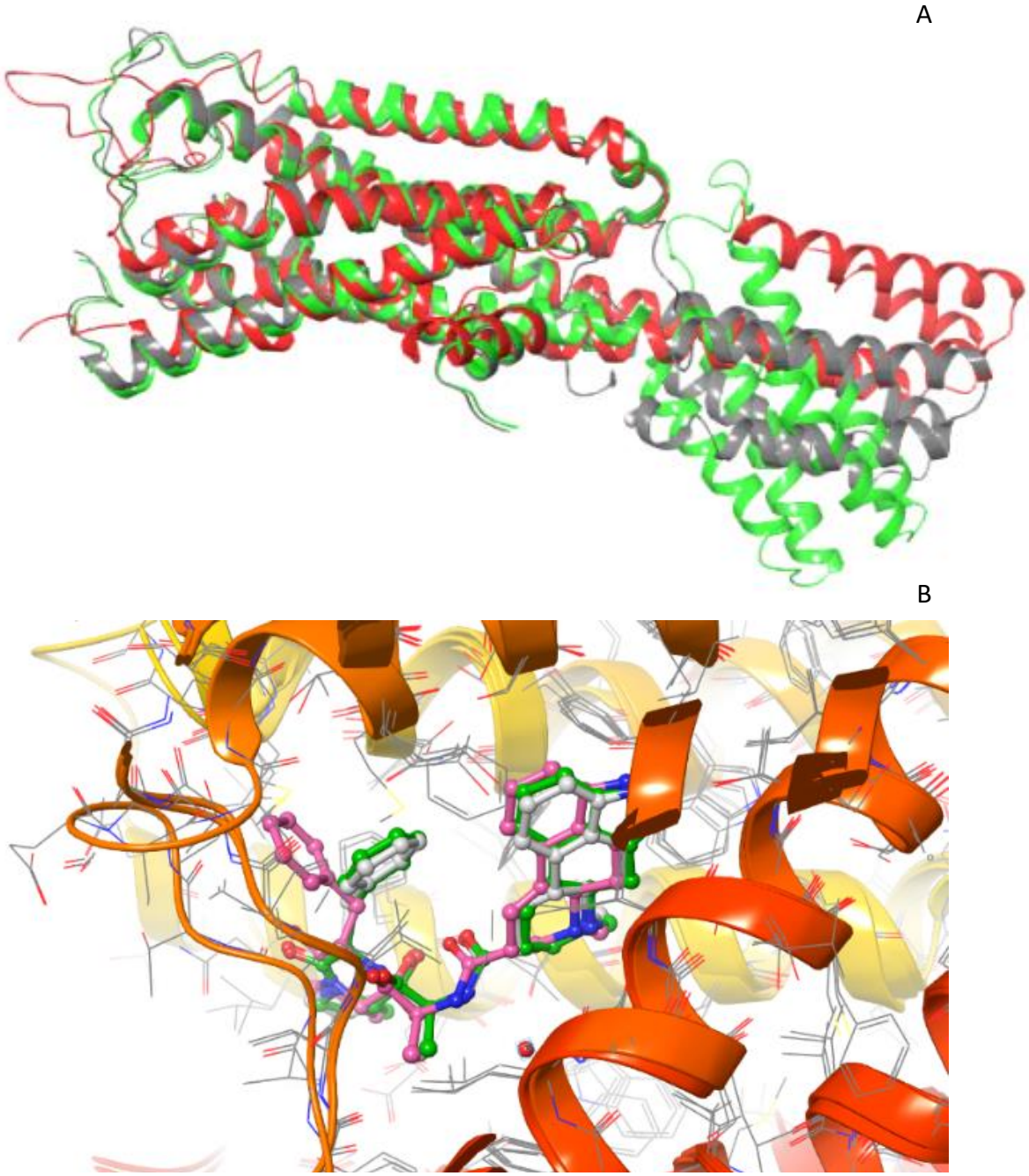


Figure 2.8 Superimposed images of 3 different active conformations of 5-HTRs with ergotamine ligand bound (4IAQ-green & 4IAR-grey) to 3 different active conformations (4NC3-5-HT2B-pink ligand). A) Superimposed structures of 5-HT1B receptors (5IAR-grey-4IAQ-green) and 5-HT2B receptor (4NC3-red) above with matching extra cellular half and differing conformations in the cytoplasmic region upon receptor activation by ligands B) perpendicular alignment of aromatic ring conformation of ergotamine ligand (pink) when overlaid from 4IAQ, 4IAR and 4NC3 (4IAQ-5-HT1B -grey ligand, 4IAR-5-HT1B -green ligand, 4NC3-5-HT2B-pink ligand). The above protein structures are processed using Maestro interface from Schrödinger. (5-HTRs used above are from online protein data bank with PDB ID: 4IAQ, 4IAR and 4NC3 and processed with Maestro from Schrödinger software platform)

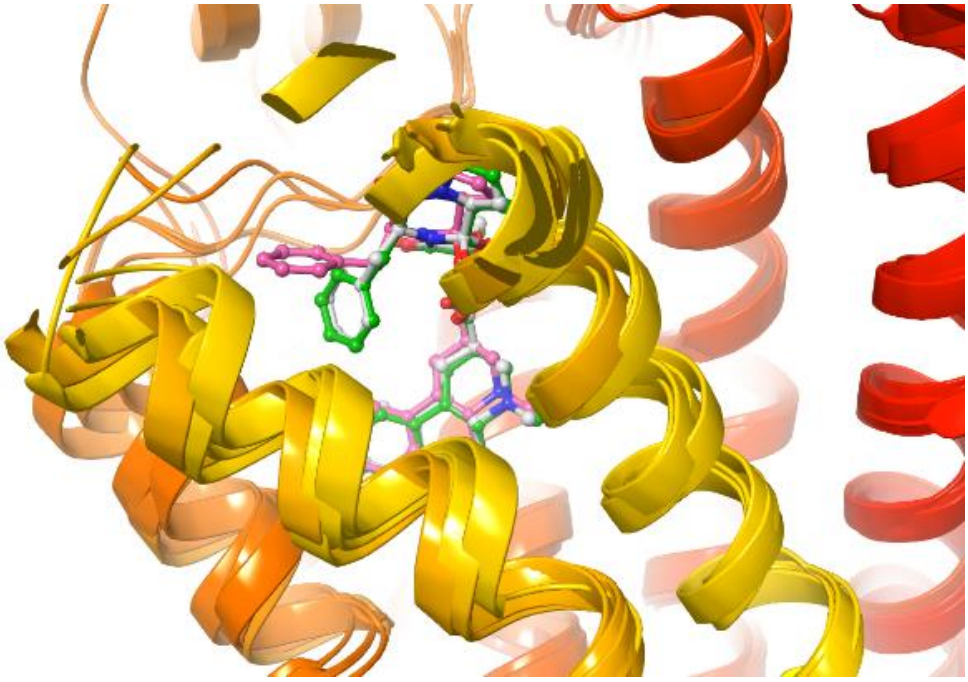


Figure 2.9 Active conformations of ligands in the LBC of 3 different receptors (4IAQ-5-HT1B -grey ligand, 4IAR-5-HT1B -green ligand, 4NC3-5-HT2B-pink ligand), indicating conformation specific different signalling properties of 5-HTRs, such as the one observed with β-AR biased signalling with some ligands.

It is known that certain 5-HTRs can stimulate cAMP activation wherein some inhibit the same via different receptor subtypes. 5-HTRs are still an extensively researched molecule, hence we lack complete knowledge of its different molecular signalling mechanisms. It is noticed that 5-HTRs, such as 5-HT2$_B$ can have ligand specific signalling where in they differ in recruiting seconday preteins such as some ligands can recruit both G-proteins and β-arrestin equally while some recruit β-arrestin selectively (Fig.2.10) (54). Hence it would be interesting to note that extended binding pocket will be crucial in influencing this selection of different signalling pathways, as the ligand conformation difference is quite apparent in 5HT1$_B$ and 5-HT2$_B$ when overlaid (Fig.5 (B & C). Hence with different anti-migraine drugs though they are successful *insilico* in establishing orthosteric and extended binding pocket interactions, it would be difficult to establish which molecular signalling pathways they can trigger in real biological systems (section 1.5.4 to 1.5.6) and this would be an interesting area to explore.

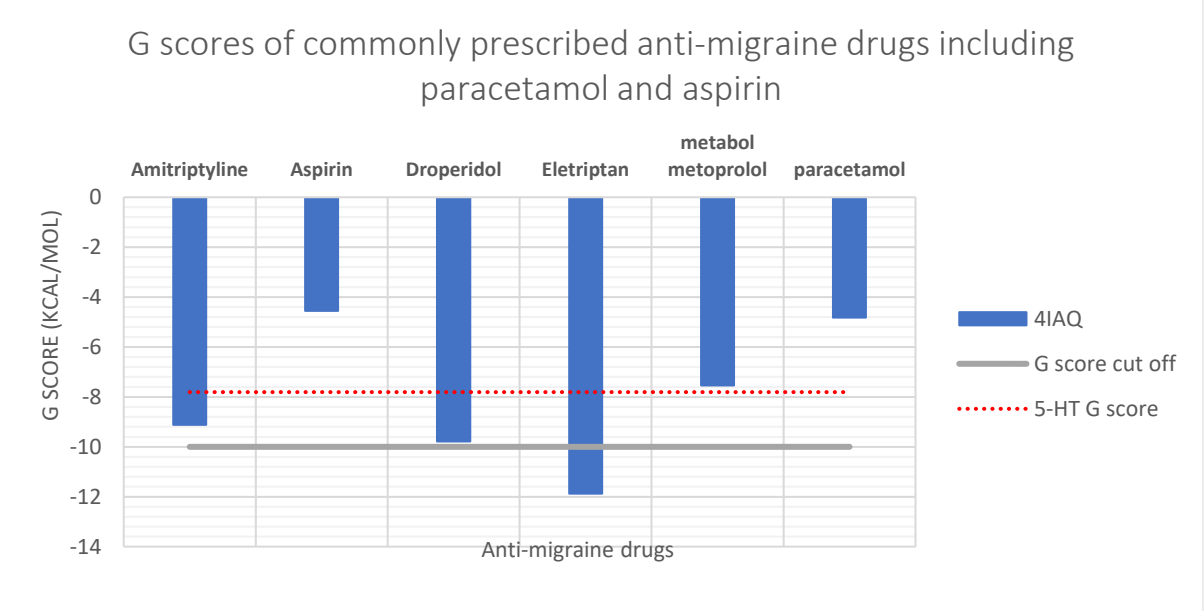


Figure 2.10 Comparison of g scores of pain killers such as paracetamol and aspirin.

	Receptor: PDB ID - Receptor subtype										
Anti-migraines/Ligands	4IAQ-5HT1B	4IAR-5HT1B	4IB4-5HT2B	4NC3-5HT2B	5AIN-5HT3	5TVN-5HT2B	5V54-5HT1B	6BQG-5HT2C	6BQH-5HT2C	6CM4-D2	5TUD-5HT2B
Active Gabapentin	-4.805	-4.974	-5.52	-5.86	-8.818	-4.453	-7.595	-6.103	-6.128	-5.88	0
Amitriptyline	-9.12	-8.39	-6.47	-10.145	-1.683	-6.741	-11.648	-8.352	-10.769	0	0
Aspirin	-4.547	-4.052	-5.195	-5.256	2.165	-4.908	-5.357	-4.401	-5.243	-4.584	0
Dopamine	-8.701	-8.194	-8.379	-8.524	-11.465	-7.541	-9.287	-8.254	-8.904	-7.714	0
Droperidol	-9.788	-8.952	-12.145	-12.235	-4.478	-9.128	-9.064	-12.186	-10.495	-7.477	0
Eletriptan	-11.879	-10.363	-6.149	-7.667	-13.933	-5.8	-10.912	-8.464	-4.874	-4.668	0
Ergotamine	-10.295	-11.826	-12.518	-12.436	0	-8.733	-10.156	-10.086	-12.801	-4.372	0
Gabapentin	-5.674	-4.884	-5.52	-5.491	-5.341	-4.454	-7.502	-6.106	-5.606	-5.783	0
Metoclopramide	-8.15	-8.195	-7.413	-8.128	-12.18	-7.101	-9.256	-8.007	-7.222	-5.956	0
Metoprolol	-7.364	-5.958	-8.193	-8.668	-11.947	-8.707	-8.4	-8.234	-6.597	-6.394	0
Nortriptyline	-9.007	-7.505	-10.626	-9.928	0	-6.746	-11.779	-8.434	-11.224	-3.303	0
Propranolol	-9.148	-8.807	-9.943	-10.29	-3.22	-9.626	-10.711	-11.119	-7.929	-7.31	0
Serotonin	-8.2	-7.414	-8.927	-8.834	-10.106	-6.466	-9.12	-9.326	-7.614	-6.975	0
Sumatriptan	-10.24	-9.82	-8.319	-9.31	-13.046	-7.69	-9.391	-9.047	-4.904	-2.357	0
Topiramate	-5.882	-5.325	-4.849	-5.779	0	-5.74	-6.785	-4.716	-6.737	-3.104	0
Verapamil	-7.506	-6.224	-7.996	-7.706	-8.722	-6.721	-9.836	-8.236	-8.25	-7.381	0
metabol D617 verapamil	-9.658	-7.528	-10.319	-8.061	-10.359	-7.854	-10.36	-9.282	-10.172	-8.579	0
metabol EHNT-Amytryptl	-9.964	-9.249	-8.018	-7.762	0	-7.153	-12.23	-11.21	-11.82	-3.644	0
metabol Nortriptyline	-8.962	-7.363	-10.626	-9.928	0	-6.746	-11.779	-8.434	-11.224	-3.303	0
metabol Norverapramil	-10.51	-8.19	-11.403	-9.102	-3.83	-8.546	-11.281	-10.6	-9.841	-6.6	0
metabol Salicycluric acid	-5.863	-5.449	-5.281	-5.482	-7.06	-6.116	-5.709	-5.546	-6.045	-4.98	0
metabol metaclopramide	-7.914	-6.203	-9.036	-8.988	-11.532	-9.394	-9.184	-9.041	-7.183	-7.141	0
metabol metoprolol	-7.547	-9.176	-8.218	-9.179	-10.157	-8.971	-9.429	-8.899	-7.432	-8.023	0
metabol propranolol	-9.528	-7.908	-10.166	-10.748	-12.046	-9.65	-11.215	-11.287	-7.973	-7.065	0
paracetamol	4.818	-6.146	-4.034	-4.273	-5.438	-4.675	-3.726	-4.434	-4.465	-4.683	0
paracetamol metabol NAPI	4.149	-4.802	-5.24	-5.177	-2.626	-5.821	-5.157	-5.832	-4.951	-4.378	0
Antagonists											
Clozapine-antagonist	-9.083	-6.849	-5.904	-6.885	0	-5.225	-10.289	-7.146	-8.23	-4.333	-7.381
Clozapine-antagonist	-7.659	-5.514	-5.367	-4.614	0	-2.686	-7.819	-6.578	-6.901	-5.006	-7.426
Cyproheptadine-antagonis	-8.377	-6.804	-4.893	-4.605	0	-6.026	-11.487	-3.295	-11.282	-3.772	-4.891
Cyproheptadine-antagonis	-7.119	-6.101	-4.147	-5.245	0	-5.198	-8.788	-5.399	-8.182	-0.843	-2.025
Loxapine-antagonist	-9.255	-7.178	-6.598	-7.116	0	-5.824	-11.109	-6.731	-8.686	-5.369	-6.737
Loxapine-antagonist	-6.068	-5.2	-4.74	-4.587	0	-5.316	-7.878	-7.341	-7.471	-6.188	-7.101
Risperidone-antagonist	-9.244	-7.662	-11.607	-10.108	0	-5.587	-10.146	-10.571	-9.91	-6.034	-10.129
Risperidone-antagonist	-8.403	-6.39	-10.355	-7.171	-2.663	-5.557	-9.518	-10.206	-8.484	-5.127	-8.916
Risperidone-antagonist	-9.493	-5.682	-8.419	-8.019	-2.114	-7.68	-8.842	-7.666	-7.678	-6.013	-7.249

Table 2.2 Best values of Glide scores of various ligands with respect to the receptors that were docked in multiple experiments. Glide scores were sensitive to conformations of a ligand when docked and different conformations in 3D had different scores, however, the best scores are selected in the above table.

Receptor: PDB ID - Receptor subtype										
Anti-migraines/Ligands	4IAQ-5H	4IAR-5HT	4IB4-5HT2B	4NC3-5HT2B	5AIN-5HT3	5TVN-5HT2B	5V54-5HT1B	6BQG-5HT2C	6BQH-5HT2C	6CM4-D2
Aspirin	-4.547	-4.052	-5.195	-5.256	2.165	-4.908	-5.357	-4.401	-5.243	-4.584
Dopamine	-8.701	-8.194	-8.379	-8.524	-11.465	-7.541	-9.287	-8.254	-8.904	-7.714
Eletriptan	-11.879	-10.363	-6.149	-7.667	-13.933	-5.8	-10.912	-8.464	-4.874	-4.668
Ergotamine	-10.295	-11.826	-12.518	-12.436	0	-8.733	-10.156	-10.086	-12.801	-4.372
Propranolol	-9.148	-8.807	-9.943	-10.29	-3.22	-9.626	-10.711	-11.119	-7.929	-7.31
Serotonin	-8.2	-7.414	-8.927	-8.834	-10.106	-6.466	-9.12	-9.326	-7.614	-6.975
Sumatriptan	-10.24	-9.82	-8.319	-9.31	-13.046	-7.69	-9.391	-9.047	-4.904	-2.357

Table 2.3 Glide scores of some selected ligands when they were docked into different receptors in multiple experiments. Negative scores indicate negative binding energy to the LBC of 5-HTR.

Results of docking scores, g scores and glide energy values from the *insilico* docking experiments are illustrated in Table 2.1, 2.2, 2.3 and 2.4. The analysed results are illustrated in Fig. 2.6, 2.7, 2.8, 2.9, 2.10 & 2.11. Molecular mimicry of antimigraine drugs to 5-HT is illustrated in Fig. 2.4, which may have influenced in their binding to 5-HTR receptors when *insilico* docking experiments were carried out and resulted in good binding scores (negative binding energy (or glide energy), g score and docking score) as illustrated in Table 2.1.

Anti-migraines/Ligands	4IAQ	4IAR
Active Gabapentin	-4.805	-4.974
Amitriptyline	-9.12	-8.39
Aspirin	-4.547	-4.052
Dopamine	-8.701	-8.194
Droperidol	-9.788	-8.952
Eletriptan	-11.879	-10.363
Ergotamine	-10.295	-11.826
Gabapentin	-5.674	-4.884
Metoclopramide	-8.15	-8.195
Metoprolol	-7.364	-5.958
Nortriptyline	-9.007	-7.505
Propranolol	-9.148	-8.807
Serotonin	-8.2	-7.414
Sumatriptan	-10.238	-9.82
Topiramate	-5.882	-5.325
Verapamil	-7.506	-6.224
metabol D617 verapamil	-9.658	-7.528
metabol EHNT-Amytryptl	-9.964	-9.249
metabol Nortriptyline	-8.962	-7.363
metabol Norverapramil	-10.513	-8.19
metabol Salicycluric acid	-5.863	-5.449
metabol metaclopramide	-7.914	-6.203
metabol metoprolol	-7.547	-9.176
metabol propranolol	-9.528	-7.908
paracetamol	-4.818	-6.146
paracetamol metabol NAPB	-4.149	-4.802
Paracetamol-Quinoneimine	-4.387	-4.533
Paracetamol-glucuronide	-6.154	-5.793
Paracetamol-mercapturic acid-N-acetyl cysteine	-4.977	-3.962
Paracetamol-sulphate	-3.677	-3.358
Antagonists		
Clozapine-antagonist	-9.083	-6.849
Clozapine-antagonist	-7.659	-5.514
Cyproheptadine-antagonist	-8.377	-6.804
Cyproheptadine-antagonist	-7.119	-6.101
Loxapine-antagonist	-9.255	-7.178
Loxapine-antagonist	-6.068	-5.2
Risperidone-antagonist	-9.244	-7.662
Risperidone-antagonist	-8.403	-6.39
Risperidone-antagonist	-9.493	-5.682

Table 2.4 Glide scores of ligands to 2 specific receptors of 5-HT1_B known by their PDB ID: 4IAQ & 4IAR.

2.3 Ligand interactions

Title	Entry ID	glide gscore	docking score	glide emodel	glide energy	glide evdw	glide ecoul	XP GScore	State Penalty	glide rmsd to input
Eletriptan	2	-11.21	-11.209	-73.235	-48.435	-43.118	-5.317	-11.21	0.0016	84.225
metabol Norverapramil	3	-10.513	-10.512	-80.257	-53.306	-46.453	-6.854	-10.513	0.0005	87.185
Sumatriptan	5	-10.238	-10.234	-68.035	-48.276	-38.276	-10	-10.238	0.0039	83.917
metabol EHNT-Amytryptl	6	-9.964	-9.96	-56.181	-41.379	-33.072	-8.307	-9.964	0.0034	83.995
metabol D617 verapamil	7	-9.658	-9.658	-46.328	-35.821	-20.806	-15.014	-9.658	0	85.587
metabol EHNT-Amytryptl	8	-9.587	-9.584	-51.039	-40.149	-30.644	-9.506	-9.587	0.0034	82.461
metabol propranolol	9	-9.528	-9.194	-57.951	-48.43	-31.936	-11.494	-9.528	0.3346	32.63
Ergotamine	13	-9.125	-8.947	-84.972	-59.729	-54.137	-5.592	-9.125	0.1781	83.218
metabol propranolol	15	-9.21	-8.876	-56.299	-42.039	-31.888	-10.151	-9.21	0.3346	82.534
metabol metaclopramide	19	-7.914	-7.913	-52.615	-38.246	-26.44	-11.807	-7.914	0.0007	82.206
Droperidol	24	-8.376	-7.264	-73.836	-47.98	-43.449	-4.53	-8.376	1.1122	88.049
Serotonin	23	-7.419	-7.419	-43.976	-31.753	-23.11	-8.643	-7.419	0	83.085

Table 2.5 Glide emodel scoring is useful to learn the favourable ligands to a given LBC investigated. As expected ergotamine is the best fit according to the glide emodel score in the above table, followed by norverapamil, eletriptan, droperidol and sumatriptan. PDB ID: 4IAQ is used for the docking results above. Natural ligand 5-HT had lower scores compared to the above-mentioned molecules and had a negative binding energy as indicated by glide energy to establish favourable interaction to the LBC of the receptor.

Glide docking experiment gives a detailed output of results as in Table 2.5, which helps to study the ligands and compare them based on different scoring parameters. If ligands are not appropriately protonated before docking experiment such as carboxylic acids should be deprotonated and aliphatic amines should be protonated, which was not done in this experiment while sketching the ligands instead used LigPrep to generate required protonation states based on pH 7 +/- 2, (Fig. 2.16). Ligands did have neutral aliphatic amines which could have improperly acted as a hydrogen-bond acceptor in the docking calculations or could occupy a hydrophobic region without incurring any penalty that XP Glide docking would have assessed if the amine group had been properly protonated. The above error could have happened in this experiment as, ligands were not screened appropriately to make sure they were protonated or deprotonated depending on the pH of the biological solution which is approximately at 7 (or 7.4 in some published trials). This is especially crucial in metalloproteins, however the proteins we studied were not metalloproteins.

AAs generally have two functional groups in them, such as an amino group and a carboxyl group with different pK_a values to each functional group. Depending on the pH of the medium where they are existing and the net charge of the functional groups they carry would give the AAs a polarity or net charge. In real biological solutions where a pH is approximately 7, the functional groups in AAs can co-exist in both a deprotonated and a

protonated ionic form depending on the pK_a of the functional groups and the net electric potential combining all the functional groups will decide the net charge of the AA. Zwitterionic forms of AAs exist at specific pH, where the net charge of all functional groups become zero, which is a state called the isoelectric point of an AA. The functional groups of AAs could exist in ionic form even during isoelectric point of an AA. The pK_a values for amines and hydroxyl groups are generally higher than the pH of biological solutions (which is approximately pH 7), and for acids such as carboxyl groups would be lower than pH 7. When pH of the medium is equal to the pK_a of the functional groups an equilibrium will exist where a deprotonated form of the AAs will be equal to the protonated form of AAs.

The ionic forms of AAs resulting from both protonation and deprotonation (Fig. 2.18), as the neutral forms of AAs are very rare, will participate in a chemical reaction with other molecules such as ligands resulting in weak reversible chemical bonds such as ionic salt bridges, hydrogen bonding, and more weaker interactions involving mild electrostatic potential differences such as van der Waals forces and pi-pi stacking forces and in some instances strong irreversible chemical bonding such as covalent bonding can also be formed which typically involves a higher binding energy such as 50 to 150 Kcal/mol. The relationship between pH and pK_a can be expressed using the Henderson-Hasselbalch equation as in Fig. 2.16.

$$pK_a = pH - \log_{10} \frac{[A^-]}{[HA]}$$

$$pK_a = -\log_{10} (K_a)$$

$$K_a = \frac{[H^+]\,[A^-]}{[HA]}$$

Figure 2.12 Relationship of dissociation constant (K_a), pH & pK_a expressed using the Henderson-Hasselbalch equation. Where the concentrations of acid base chemical species expressed as HA, and their dissociated ionic forms A^-, and H^+ are in equilibrium in a buffer solution, and the dissociation constant as K_a.

The glide energy values from this experiment are indicative of weaker molecular interactions between ligands and 5-HTRs, hence the ligand interactions established after

the docking experiment would give reversible molecular bonding such as salt ionic bridge or hydrogen bonding interactions, and more weak interactions such as van der Waals interactions & pi-pi stacking interactions. These interactions can be studied in detail using ligand interaction diagrams.

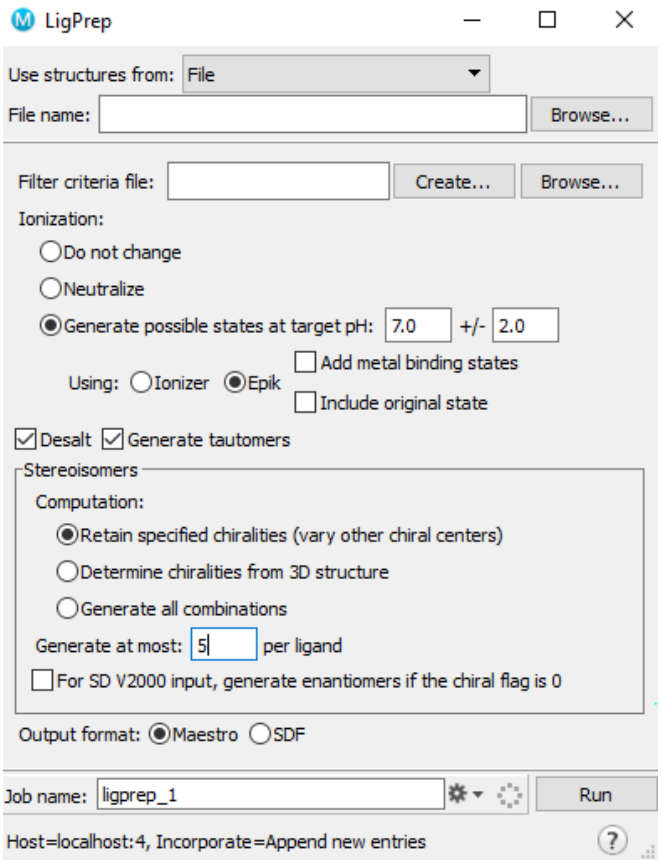
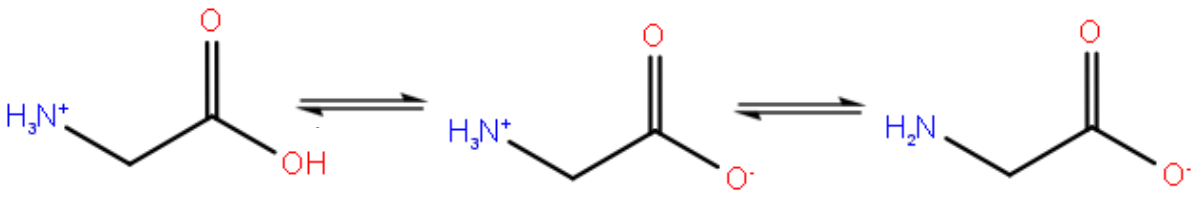


Figure 2.13 LigPrep window where ionization state was selected to generate possible protonation states for ligand to facilitate docking with appropriate energy levels.



Protonated state at low pH Zwitterionic state at pH 7 Deprotonated state at high pH

Figure 2.14 Example of a protonated and deprotonated forms of an AA. Protonated state at low pH refers to the functional groups having a higher pK_a than the pH of the solution medium and vice versa when it is deprotonated at high pH, i.e., the functional groups having a lower pK_a than the pH of the solution medium.

Some of the ligand interactions from the docking experiment carried out, using the 5-HTR template 4IAQ is illustrated using the docking results for selected molecules in the following sections.

2.3.1 Receptor details: 4IAQ

The molecular details of the receptor protein at the time of download are;

- Total Structure Weight (Molar Mass): 46100.71
- Atom Count: 2795
- Residue Count: 403
- Unique protein chains: 1
- Molecule: Chimera protein of human 5-hydroxytryptamine receptor 1B and E. Coli soluble cytochrome b562
- Protein: 5-HT1_B (serotonin receptor) with bound ergotamine
- Mutations: 4
- Gene Names: cybC, HTR1B (HTR1DB)
- pH: 8.7
- Temperature: 293K
- Ligand interaction: Dihydroergotamine

However, the above details were changed slightly after protein preparation process using Maestro as follows;

A

SELECTED	0 atoms	0 residues	ATOMS	2800	CHAINS	1	ENTRIES 1
DISPLAYED	2800 of 2800	373 of 373	RESIDUES	373	MOLS	10	CHARGE -8

B

SELECTED	0 atoms	0 residues	ATOMS	6345	CHAINS	1	ENTRIES 1
DISPLAYED	6345 of 6345	394 of 394	RESIDUES	394	MOLS	3	CHARGE 2

Table 2.6 Screen shot tables from Maestro window displaying details of residues and atoms of the receptor 4IAQ. A) The details as per Maestro before processing B) The details as per Maestro before processing. The missing atoms were added by the software at the time of processing changed from 2800 atoms to 6345 atoms and the number of residues changed from 373 to 394. Hence the information obtained from the PDB website and Maestro is different, and since Maestro is an advanced software the above information may be more reliable regarding the receptor details.

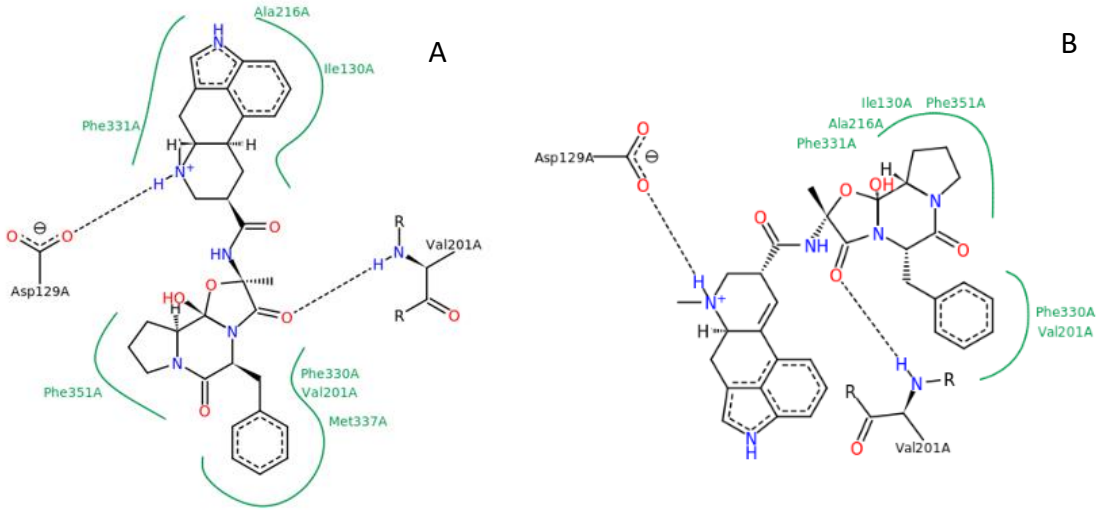
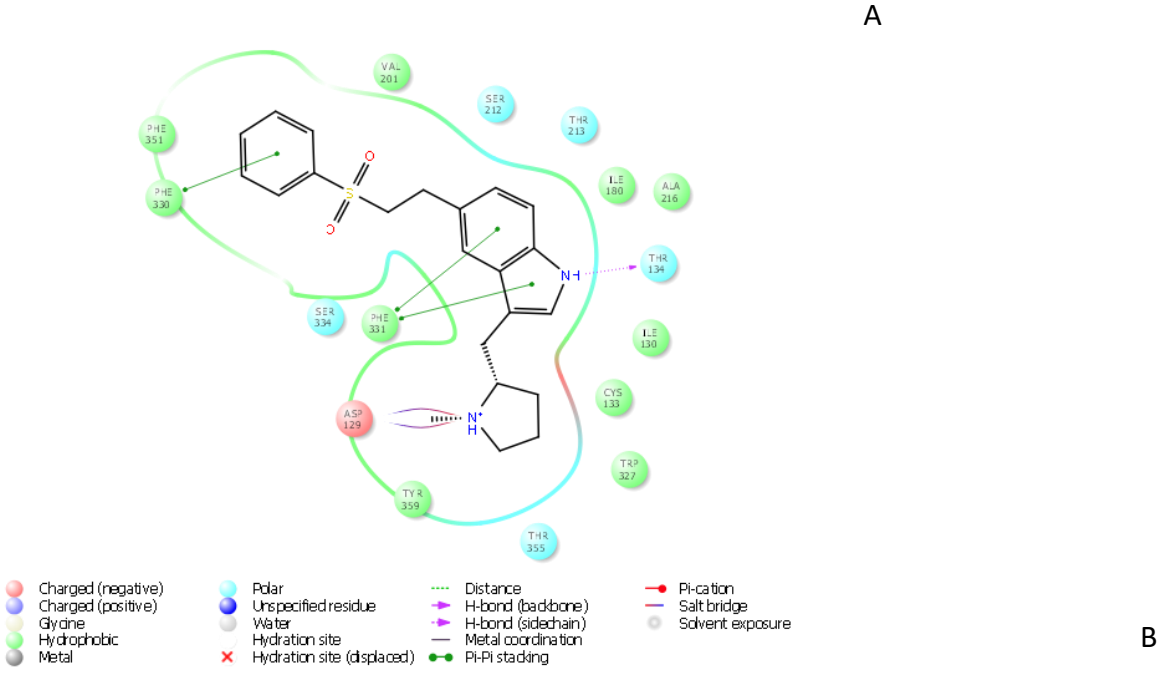


Figure 2.15 A) Ligand interactions of dihydroergotamine with receptor 4IAQ. B) Ligand interactions of ergotamine with receptor 4IAR. These Images are downloaded from online protein data bank web site, where the x-ray crystallographic receptors 4IAQ and 4IAR are listed in bound active conformations (77, 81). The above interactions are indicative of forming a salt bridge and a hydrogen bond at the LBC.

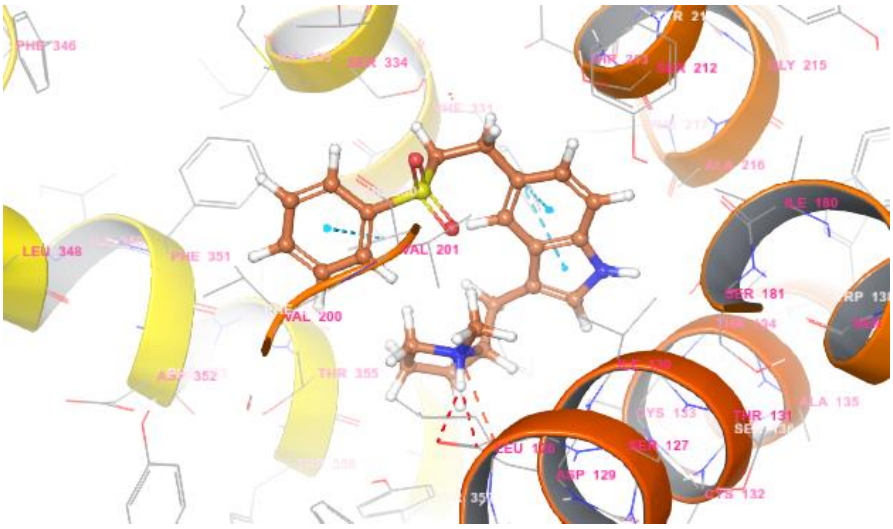
2.3.2 Ligand interactions: Eletriptan



Title	Entry ID	glide gscore	docking score	glide emodel	glide energy	glide evdw	glide ecoul	XP GScore	State Penalty	glide rmsd to input
Eletriptan	2	-11.21	-11.209	-73.235	-48.435	-43.118	-5.317	-11.21	0.0016	34.225

Figure 2.20 continues to next page (108)

C



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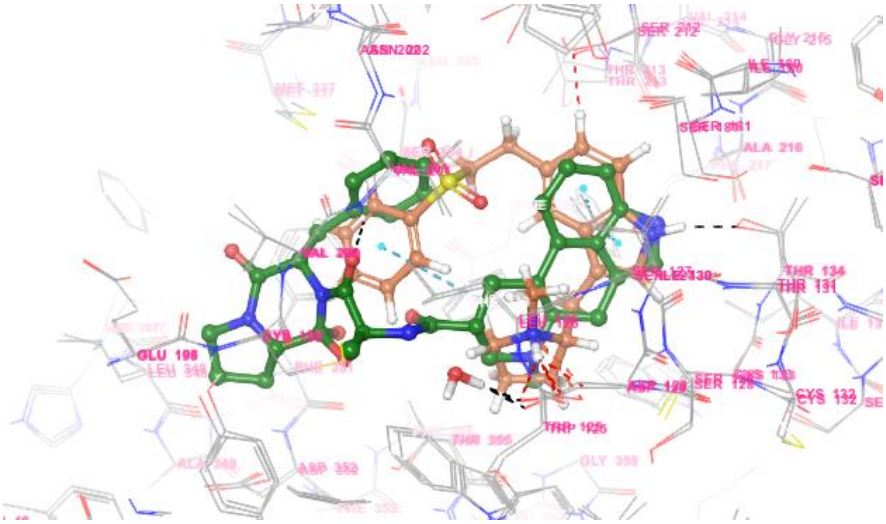


Figure 2.16 Above images of Eletriptan docked at the LBC of 4IAQ using Glide software from Schrödinger. A) Ligand interaction diagram of eletriptan at the LBC of 4IAQ. B) Summary of results obtained for eletriptan post docking experiment with the receptor. C) Eletriptan establishing interactions at the LBC of 4IAQ post docking experiment. D) Dihydroergotamine overlaid to eletriptan at the LBC using Glide to display the core of ligand docking is same for both the ligands.

Eletriptan as a drug is generally prescribed for the acute treatment of migraine. I used this drug for the docking experiment to study its interactions.

Eletriptan was able to establish successful interactions with the receptor post docking, the details of interactions were (Fig. 2.21);

- H- bond to THR 134, bond distance 1.8 Å, acceptor angle 167^0
- Salt bridge interaction to ASP 129, bond distance 2.5 to 2.8 Å

- Pi-Pi stacking distance to PHE 330 was 5.4 Å, and to PHE 331 was 5.1 to 5 Å

Rigid receptor conformation was maintained to receptor core within tolerance of 0.1 to maximum 1 Å.

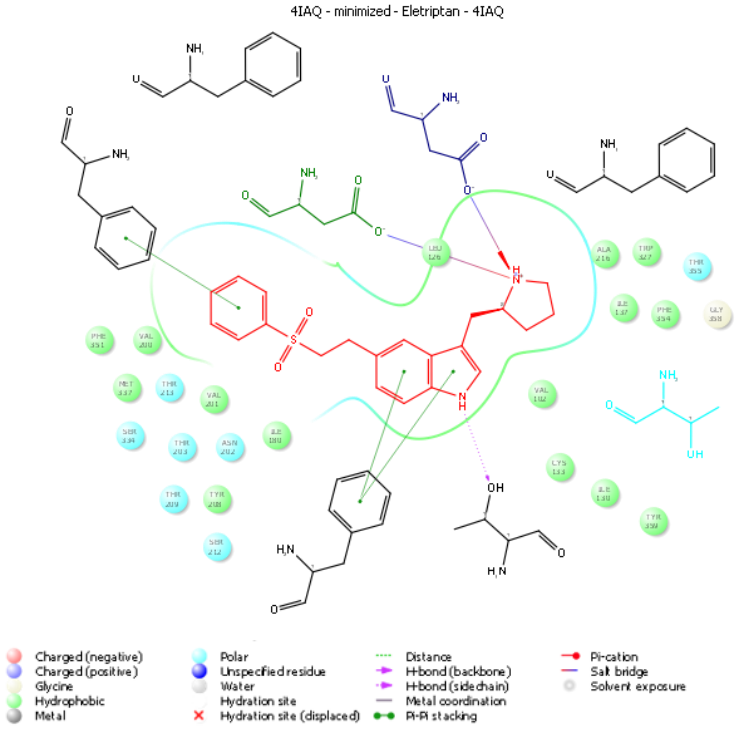


Figure 2.17 Expanded ligand interaction diagram of eletriptan at the LBC.

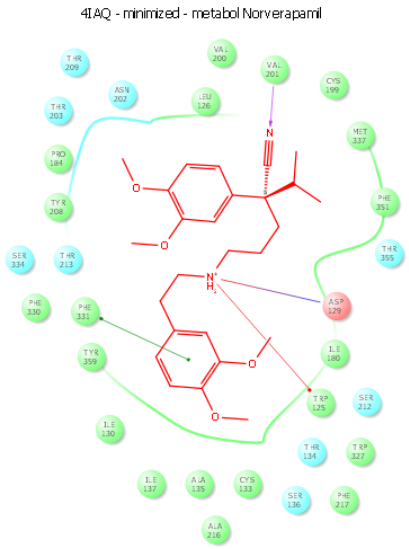
2.3.3 Ligand interactions: Norverapamil

Norverapamil is an active metabolite of verapamil, known as a calcium channel blocker mainly used in the treatment of hypertension. Verapamil is also prescribed for the treatment of migraine to relieve the symptoms of hypertension. Norverapamil was successfully docked into the receptor (4IAQ), with interactions as below (Fig. 2.22);

- H- bond to VAL 201, bond distance 2.1 Å, donor angle 122^0
- Salt bridge interaction to ASP 129, bond distance 4.2 Å
- Pi-Pi stacking distance to PHE 331 was 5.2 Å
- Pi-cation interaction distance to TRP 125 was 6.3 Å

A

Title	Entry ID	glide gscore	docking score	glide emodel	glide energy	glide evdw	glide ecoul	XP GScore	State Penalty	glide rmsd to input
metabol Norverapamil	3	-10.513	-10.512	-80.257	-53.306	-46.453	-6.854	-10.513	0.0005	37.185



B

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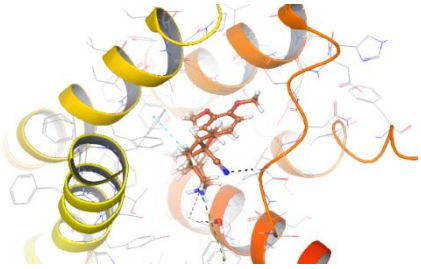


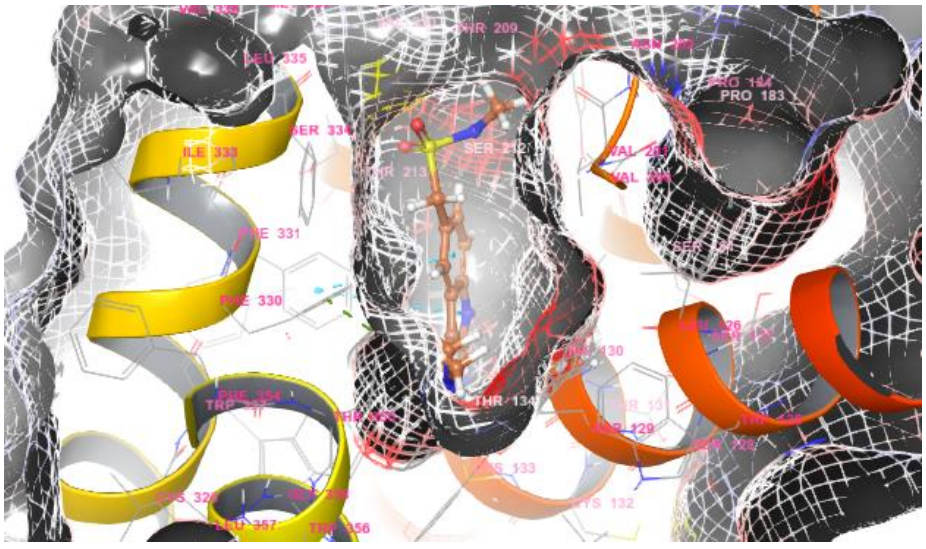
Figure 2.18 Screen shots of nor verapamil being docked as ligand at the LBC. A) Summary of results obtained for Norverapamil post docking experiment with the receptor. B) Ligand interaction diagram with arrows and straight lines indicating the contacts with the receptor. C) Pose view of the docked ligand at the LBC.

2.3.4 Ligand interactions: Sumatriptan

Sumatriptan is used as a migraine specific medication. The details of this molecule used as a ligand for the docking experiment is as follows;

A

Title	Entry ID	glide gscore	docking score	glide emodel	glide energy	glide evdw	glide ecoul	XP GScore	State Penalty	glide rmsd to input
Sumatriptan	5	-10.238	-10.234	-68.035	-48.276	-38.276	-10	-10.238	0.0039	33.917



B

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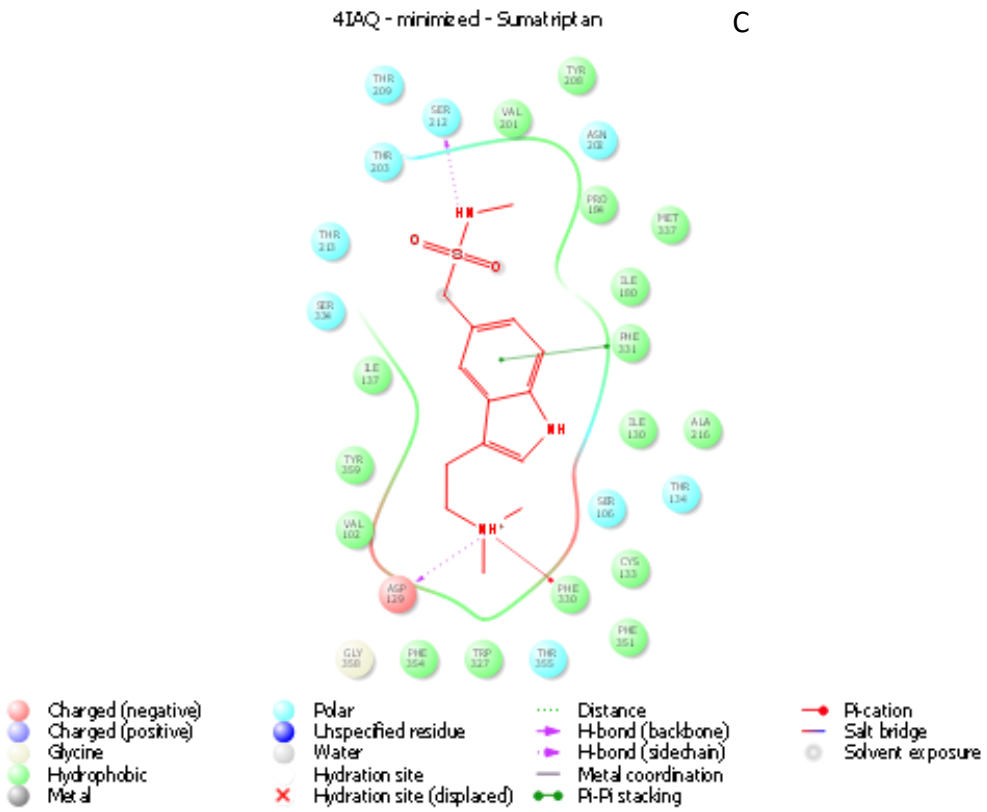


Figure 2.19 Ligand interaction results of sumatriptan when docked to 5-HTR (PDB ID:4IAQ) A) Various scoring details of sumatriptan using Glide XP docking experiment B) Topographical mesh view of the ligand binding domain of the receptor with the ligand (in ball and stick representation) docked to the LBC. C) Ligand interaction diagram of sumatriptan at the LBC of 4IAQ receptor.

Sumatriptan interacts with the receptor (Fig. 2.23) via;

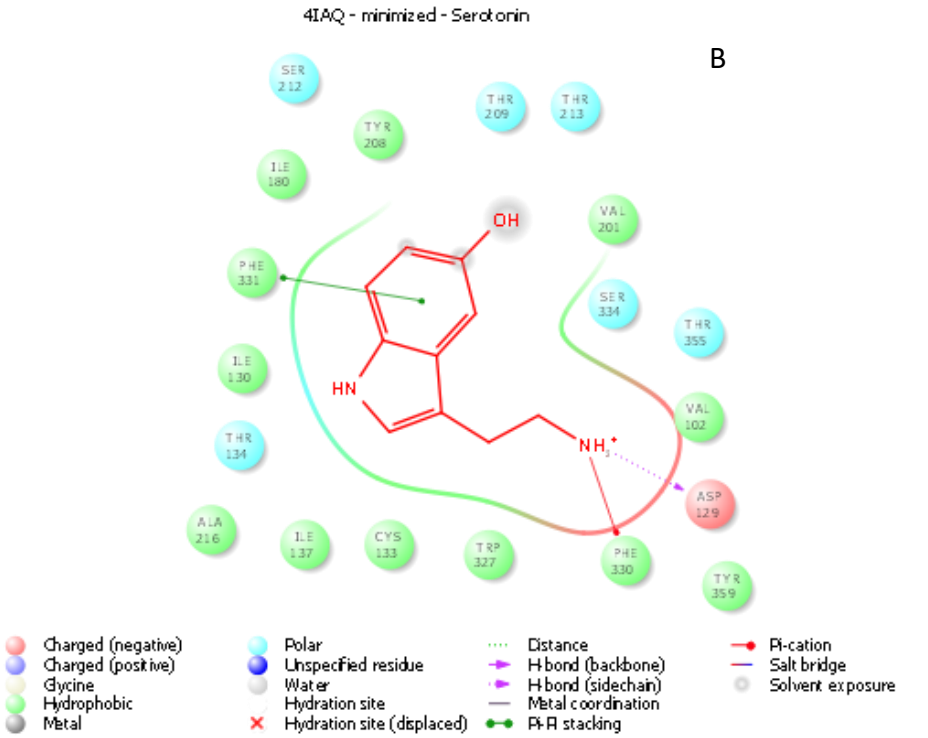
- H- bond to ASP 129, bond distance 1.7 Å, donor angle 122^0
- H- bond interaction to SER 212, bond distance 2.1 Å
- Pi-Pi stacking distance to PHE 331 was 5.1 Å

2.3.5 Ligand interactions: Natural ligand (Serotonin)

In the absence of an active natural ligand bound conformation to study the receptor, the dihydroergotamine bound active conformation of the receptor gives an idea how a ligand would interact and possibly give some indication to compare the active form with an inactive conformation if generated.

A

Title	Entry ID	glide gscore	docking score	glide emodel	glide energy	glide evdw	glide ecoul	XP GScore	State Penalty	glide rmsd to input
Serotonin	23	-7.419	-7.419	-43.976	-51.753	-23.11	-8.643	-7.419	0	33.085



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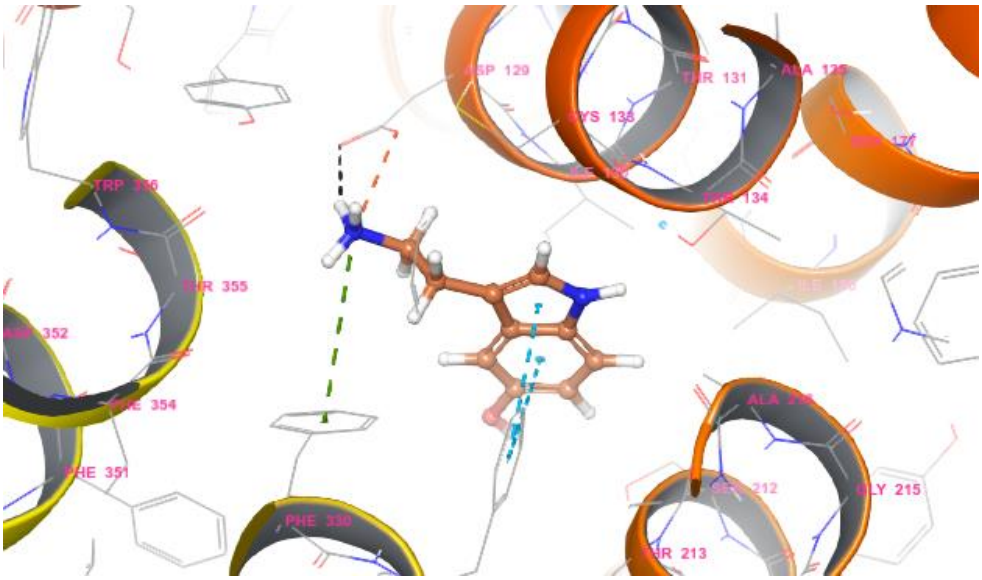

Figure 2.20 Above images of 5-HT post docking experiment results with 5-HTR (PDB ID: 4IAQ) A) Results table. B) Ligand interaction diagram. C) Pose view of the ligand at the LBC of the receptor.

5-HT interacts with the receptor (Fig. 2.24) via;

- H- bond to ASP 129, bond distance 2.1 Å, donor angle 92^0
- Pi- cation interaction to PHE 330, bond distance 5.0 Å
- Pi-Pi stacking distance to PHE 331 was 5.3 Å

We may now know that ASP 129 interaction from the orthosteric binding pocket of the receptor is crucial in recognising the ligand, as the dihydro ergotamine bound structure of 4IAQ involved interaction with ASP129. The extended binding pocket interaction involving Val 201 is missing with natural ligand interaction which may have contributed to low glide scores with 5-HT. This indicates the fact that 5-HT may have an on and off interaction at the orthosteric pocket whereas the dihydroergotamine had more stability at the LBC due to its interaction with extended binding pocket. He orthosteric pocket comprised mainly hydrophobic residues of Val 102, Tyr 359, Phe 330 and negatively charged Asp 129. Polar residues comprised of Thr209, Thr355 and Ser 334 were also part of the LBC.

Chapter 3 Discussions

3 Discussion

3.1 Validity of the *insilico* experiments

Scoring functions used by the software Glide XP, is based on enforcement of the physical and chemical principles of science to a great degree. The scoring methods are reliable due to its reproducibility and optimised evaluations of the scoring function for correctly docked compounds. However, the *insilico* results need to be validated with reproducibility of results in real biological experiments due to the possibility of errors in the absence of representing the real biological environment during its testing phase. However, with improved adaptation using the data from real biological systems with respect to the kinetics and the molecular dynamics energy of the participating molecules in a receptor, the interactions can be reproduced and studied for accuracy of results. The following possibility of errors and limitations need to be considered in validating the accuracy of the docking results;

- Errors in binding mode predictions
- Errors in accuracy of computational algorithms for docking and scoring results
- The algorithms for initial screening and energy minimization are not yet fully optimized
- Errors in correctly finding docked poses and rejecting false positives during screening processes
- Complicated user training modules and exhaustive details in running the software
- Not very user friendly due to many ambiguities to operations and procedures
- Lack of enough data to support the validation of *insilico* results from in vitro to in vivo results by having a comparison of experiments with real biological set up to relate to the results.
- Lack of receptor flexibility in studying the helical rearrangements post successful docking.
- Receptor conformation is rigid in the experiment, which is specific for the previously docked ligand and in real biological environment due to potential energy

differences between different ligand interactions the desired receptor conformations are unknown, hence even though the docking results are indicative of a successful binding, will change in real setting as the receptor molecules are highly dynamic and may interact differently with different conformations.

- Glide tests several possible conformations for a given ligand, to the given rigid conformation of the ligand.
- Limited flexibility can be applied by having a rigid back bone conformation for the receptor and some sidechain residues can be made rotatable, this may give some favourable docking scores for the docked ligand.

Having considered all the possible limitations, the benefits outweigh the limitations; hence Glide is a good tool to explore, learn & evaluate the possibilities in predicting the molecular interactions and designing a suitable drug or a molecule. The binding energy calculations are very detailed (as discussed in section 1.10), and the scoring functions (section 2.3) are highly useful in predicting a successful binding in docking experiments. Some of the scoring functions that were considered for evaluating the docking experiments are already listed in the results chapter (section 1.10, 2.3 and 2.4). We have compared the Gscore results of a set of anti-migraine drugs post docking in the results section 2.3. All ligands tested had a negative Gscore and negative binding energies for that specific conformation of receptor and ligand. However, the chances of achieving this specific conformation in real biological solution is not known, for this we must do experiments in biological mediums.

Lower the Gscore of a ligand, would mean more successful in binding to the receptor for that specific conformation of a receptor, many of the anti-migraine drugs tested including eletriptan, ergotamine, and sumatriptan had Gscores lower than the natural ligand 5-HT, indicating a better binding possibility to 5HTRs. Of all the ligands tested glide energies were lowest for Eletriptan and Ergotamine, and docking scores were also lowest for these two along with sumatriptan. However, at this stage we can't predict how likely they behave in real biological systems and will the same receptor and ligand conformations will be achieved.

The simplest mechanism of GPCR activation is based on a two-state conformational selection model of receptors, whereby the receptor exists in an equilibrium between active and inactive states; agonists shift the equilibrium toward the active, whereas inverse agonists shift the state to the inactive state (75). There could be many intermediate inactive and active conformations possible for GPCRs (54, 56-58, 73-75, 82), hence knowing all the molecular features of active and inactive state conformations will help in designing a drug suitable to avoid off targeting. Signalling changes during active and inactive states can be measured using laboratory-based monitoring methods, which will be discussed later in this section.

The research has met its objective in completing the *insilico* docking experiments using the anti-migraine drugs as ligands to 5-HTRs for the given specific receptor conformation.

3.2 Comparison of molecular structures of anti-migraine drugs (triptans & non triptans) with 5HT/DA and their structural analogy

As illustrated in Figure 2.4 and in section 2.3, popular antimigraine drugs such as ergotamine, eletriptan and sumatriptan had a striking structural analogy to 5-HT, this led to the understanding that anti-migraine drugs might be mimicking 5-HT in the serotonergic synapses and might activate some of the 5-HTRs on the post synaptic nerves, which might be contributing to its efficacy in migraine.

3.2.1 Research hypothesis in relation to the structural analogy concepts to tyramine

In section 1.7, my research hypothesis stated that molecular mimicry of tyramine to DA could be implicated in the form of dyshomeostasis involving DA and 5-HT, and an assumption is stated as increased DA availability in the brain could be implicated in the development of Migraine. Some dietary sources of AAs contribute to increased availability of tyramine in the system, or the brain might be able to synthesise which is unknown. Thus, non-neurotransmitter monoamines from the system, such as specific dietary AA

(section 1.4 from chapter 1 & Fig 1.1) may mimic monoamine neurotransmitters besides the other genetically predisposed factors and contribute to a lower threshold to migraine specific triggers. However, their availability in brain is limited to crossing the BBB, and tyramine is known to be present in the brain. Thus, a migraine response may be activated and that anti-migraine drugs which mimic serotonin is partly able to correct this dyshomeostasis by activating certain 5-HTRs and able to relieve the symptoms. To prove this assumption, *insilico* docking experiments (using Glide XP software) were carried out with x-ray crystallographic structures of ergotamine bound active 5-HTRs and anti-migraine drugs, to dock in a related manner by replacing the bound ligand. The results were analysed using the docking results to assess the possibility of activating 5-HTRs by anti-migraine agents.

Section 1.3.6 & section 1.4.7 have given an understanding of the monoaminergic system, their role in pain modulation, and especially serotonergic system which acts via on or off mechanism to regulate various aspects of neurogenic pain modulation. This concept of pain matrix and the role of monoaminergic system probably has a role in the symptoms of (pulsating headaches) migraine, which needs further investigation.

3.3 Do the results from docking experiments indicate the possibility of anti-migraine drugs activating 5-HTRs?

The *insilico* docking experiment results from section 2.3 and Table 2.1 gives a detailed analysis of the possibility of anti-migraine drugs activating 5-HTRs. The negative binding energies are indicative of the binding possibilities, however whether this interaction is enough to activate the receptor is a difficult question, considering the limited data available on the 5-HTRs. More so because the active conformation dynamics of the receptor is restricted due to the rigid receptor *insilico* docking. As the receptor conformation is best suited to the already bound ergotamine (ERM) ligand. Considering these limitations, the best possible approach to analyse the results would be;

- To generate an *insilico* data of how a natural ligand would interact in the given rigid receptor conformation.
- Compare the Ligand interactions of anti-migraine drugs to the natural ligand. interactions and the interactions of the already bound ligands from 4 IAQ or 4 IAR
- Study the binding energies to relate to the ligand interactions.

Based on the above discussed criteria, 5-HT had only an orthosteric LBC interaction while the ERM had both orthosteric and extended binding pocket interactions, hence the docking scores were lower with 5-HT and higher with ERM. The key residue involved in the orthosteric binding pocket was a negatively charged Asp 129 and other mixed hydrophobic or polar residues. This makes the natural ligand most likely to be involved in a salt bridge interaction in real biological scenario since the amino groups will be most likely protonated, and the hydrophobic pocket will not like a polar state and will engage the ligand in a more weaker salt bridge interaction with a longer bond distance than a hydrogen bond. The anti-migraine drugs were successful in most instances in establishing an orthosteric binding pocket interaction due to the amino group present in them and some interacted to the extended binding pocket like the ERM, resulting in a good score overall (high score of negative value) compared to 5-HT. Of all the anti-migraine drugs tested the best suited based on the scoring parameters were indicative of ergotamine, eletriptan and sumatriptan.

In section 1.2.3 meta-analysis data of migraine treatment responses shows eletriptan and sumatriptan were successful in giving good treatment response.

3.3.1 Interactions critical in ligand recognition

Comparing the interactions of natural ligand to ERM, some critical differences are in the extended binding pockets of 5-HTR subtypes. The orthosteric binding pocket seems to be common in all 5-HTR subfamilies differing only in the extended binding pocket. There is a 3 Å outward shift at the end of Helix V, when we compare the extended binding pockets of 5-HT1$_B$ and 5-HT2$_B$. This difference is key to subtype selectivity in ligand recognition among all 5-HTR subtypes.

Some of the receptor interactions critical for the LBC stability are;

- Disulphide bridge between helix III and ECL2 (Cys 122 & 199 in 5-HT1$_B$ and Cys 128 & 207 in 5-HT2$_B$) is conserved to all 5-HTRs. This disulphide bridge stabilises the extracellular loop 2 over the ligand binding pocket near the extended binding pocket.

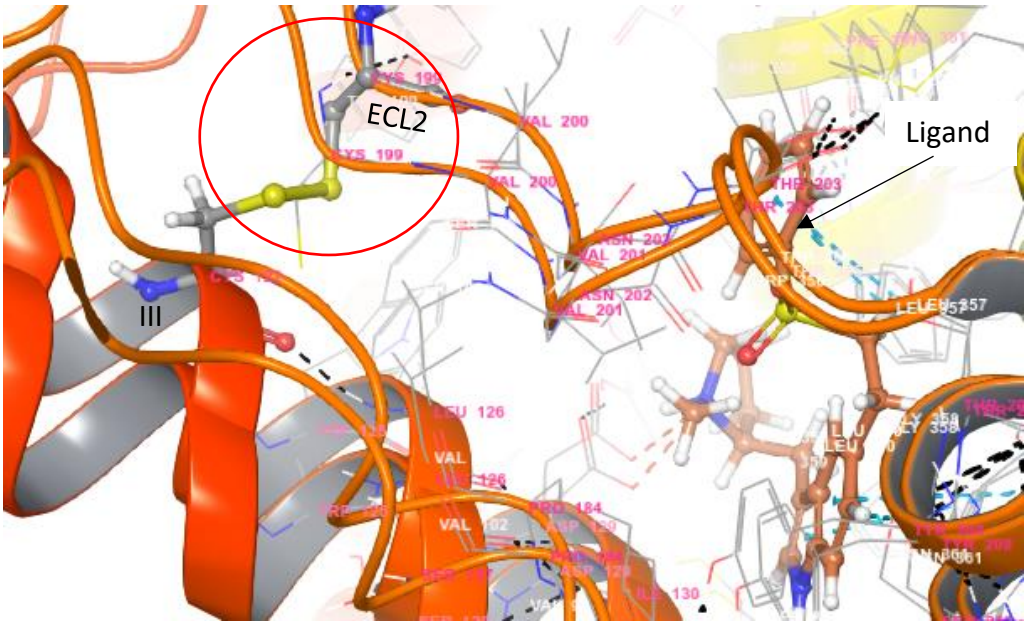

Figure 3.1 LBC of superimposed images of 4IAQ and 4IAR. The disulphide bridge (in yellow ball and stick representation) between Cys 199 and Cys 122 highlighted in red circle, helps stabilizes the LBC in position as shown closer to the ligand (in ball and stick representation, marked with an arrow label) in the above image.

- Hydrogen bonding between Asp 352 (a ligand binding residue) and Tyr 40 (close to N terminus), indicating role of N terminus in ligand recognition in 5-HT1$_B$ (Fig. 3.2)

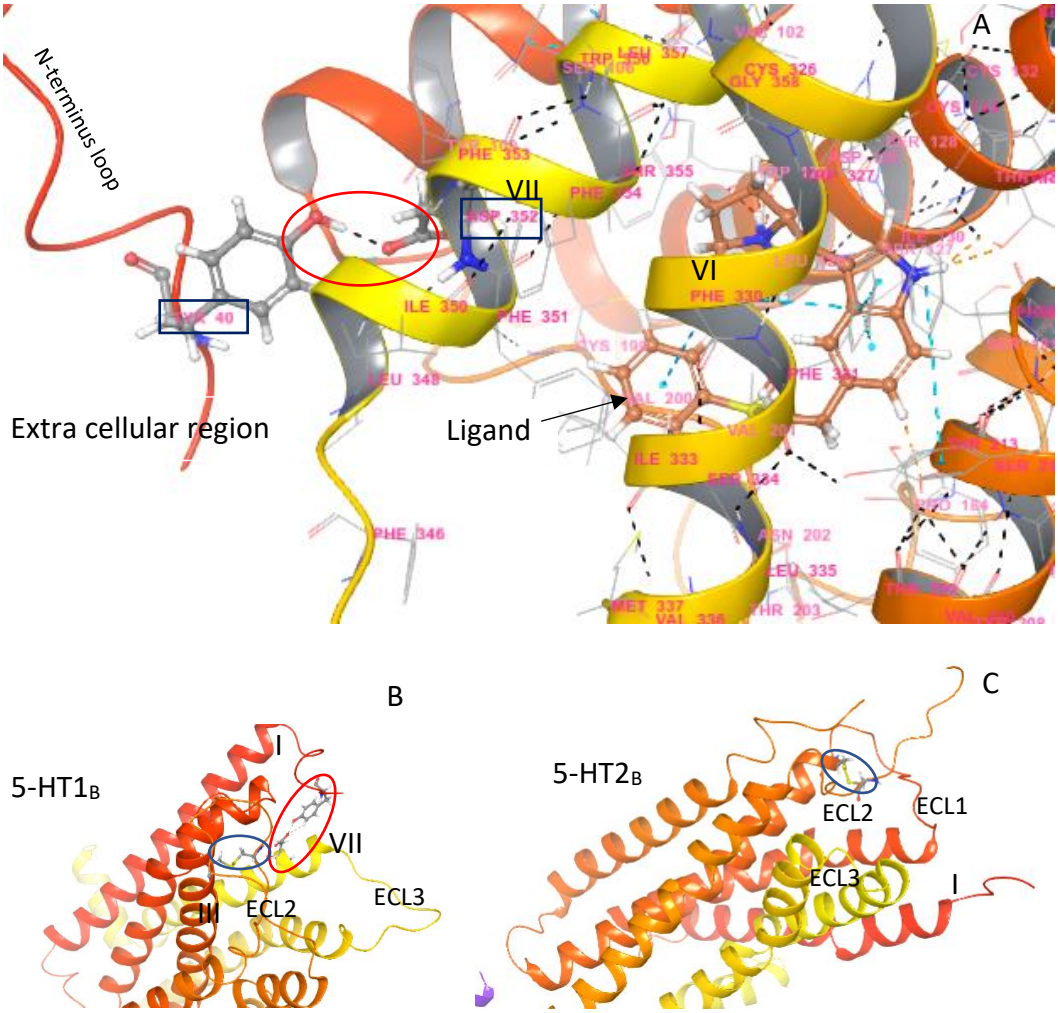

Figure 3.2 A) Tyr 40 from part of N-terminus loop (in ball and stick representation) interacting with Asp 352 (labels in black square boxes) from helix VII via a hydrogen bonding (highlighted inside red circle as black dotted line) close to the extended binding pocket of 5-HT1$_B$, indicating the role of N-terminus in ligand recognition which is not observed with 5-HT2$_B$. B) Extracellular regions of 5-HT1$_B$ with red circled area depicting the N-terminus loop interaction residue from helix VII and the yellow circled area highlighting the disulphide bridge from ECL2 to helix III. C) Extracellular region missing N-terminus loop interaction from helix I in 5-HT2$_B$, and highlighting the yellow circled area of disulphide bridge common to all 5-HTRs.

- Hydrogen bonding between Asp 129 and Tyr 359 contribute to maintaining the narrow orthosteric binding pocket.

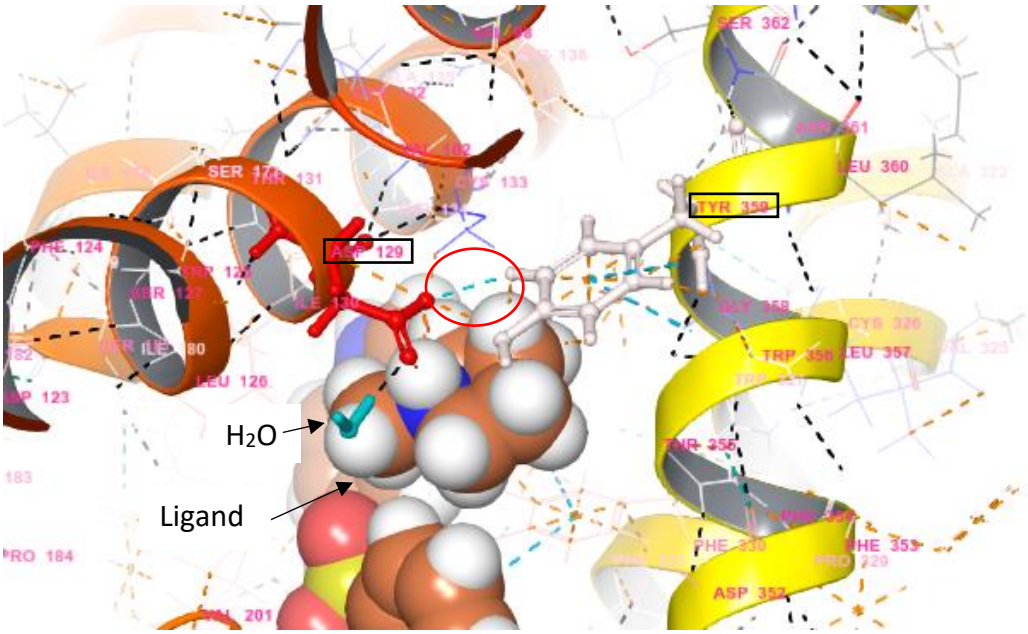

Figure 3.3 The hydrogen bonding (displayed as teal coloured dotted line inside the red circle) between Asp 129 and Tyr 359 (labelled as black square boxes with residues in ball and stick representation) in the narrow orthosteric binding pocket, where the ligand is displayed in CPK representation. Asp 129 also interacts with the amino group of the ligand.

- The 3 extra cellular loops (ECL) between the seven transmembrane helices of the receptor are displayed as ECL1, ECL2 and ECL3 in Fig. 3.4. The Ligand binding hydrophobic pocket of the receptor mostly formed by residues such as, Ser 212, Ala 216, Thr 134, Cys 133, Phe 330, Phe 331, Trp 327, Ile 130, Asp 129, Tyr 359, Asp 352, Thr 355, Phe 351, Trp 125, Leu 126, Val 200 (ECL2), Val 201 (ECL2), Thr 203 (ECL2), Thr 209, Ser 334, Met 337, & Tyr 40 (N-terminus loop) are defined with a ball and stick representation in Fig. 3.4. These residues may differ between varying 5-HTR subtypes at the extended binding pocket. Other published reports have shown similar results of 3 Å distance (Fig. 3.6) between more closer residues such

as in helix V in extended binding pockets and are found to be broader in 5-$HT1_B$ than 5-$HT2_B$ due to outward shift of helix V (54).

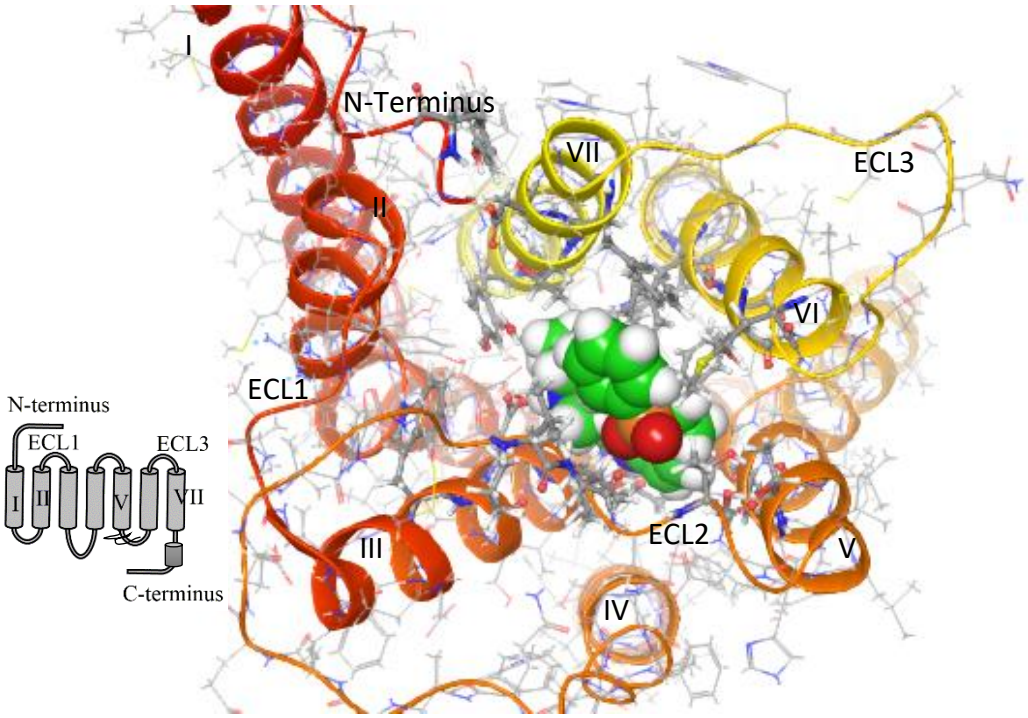

Figure 3.4 Topographical view of the LBC image from Maestro along with a cartoon representation of the GPCR receptor on the left (drawn using Chemdraw software) depicting the seven transmembrane helical structure of the receptor with inbuilt labelling of some helices and extra cellular loops. Residues in ball and stick representation define the hydrophobic LBC region of the receptor 4IAQ, while the ligand is represented in CPK form.

- The differences in binding pockets of t he2 different 5-HTRs were examined using the quick align method in Maestro. It was noticed that the Leu 46 in helix I of 5-$HT1_B$ is displaced by 9.26 Å (Fig. 3.5) compared to the similar helix (Leu 54) of 5-$HT2_B$, hence the extracellular regions of 5-HTRs may differ by more expanded or contracted binding pockets depending on their subtypes, as the replacing residues can be different in respective helices. This difference could be studied with more flexible docking experiments to understand the receptor subtype selectivity to help design more target specific drugs to avoid off target activation of the receptor

subtypes which could be one of the reasons of unwanted side effects with many drugs resulting in their withdrawal.

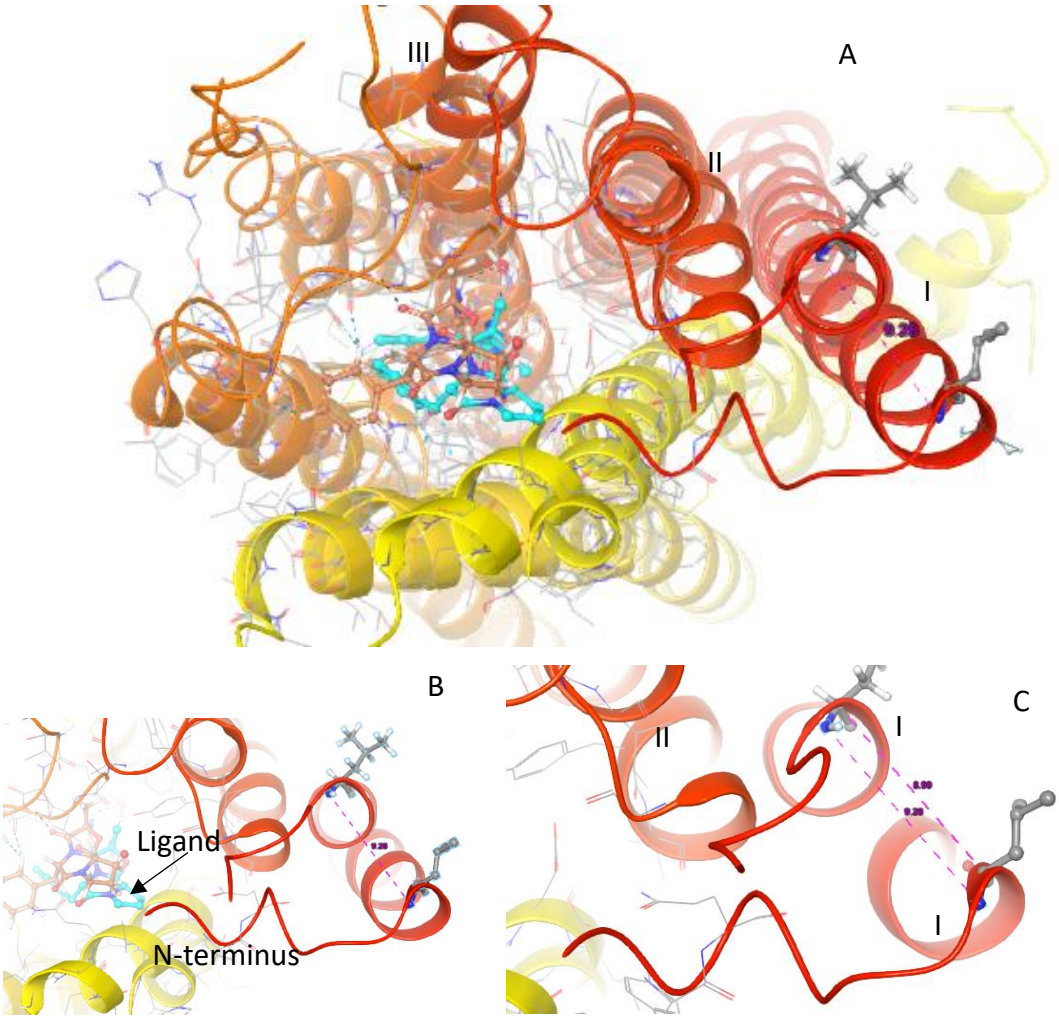

Figure 3.5 Topographical view of the quick aligned receptors of 5-HT1$_B$ and 5-HT2$_B$. A) Residues (Leu 46 of 5-HT1$_B$ & Leu 54 of 5-HT2$_B$) from helix I of the two different 5-HTRs in ball and stick representation shows the distance between the nitrogen atoms as 9.26 Å. B) Enlarged view of the helix 1 of the aligned receptors, showing part of N-terminus loop moving closer to the binding pocket indicating a role in recognising the ligand. Both the ligands from the aligned receptors are displayed in ball and stick representation indicating orientation is slightly different in both receptors as the binding pocket is more expanded in one than the other. C) The distance measured between the α-carbons of the same residues is 8.99 Å.

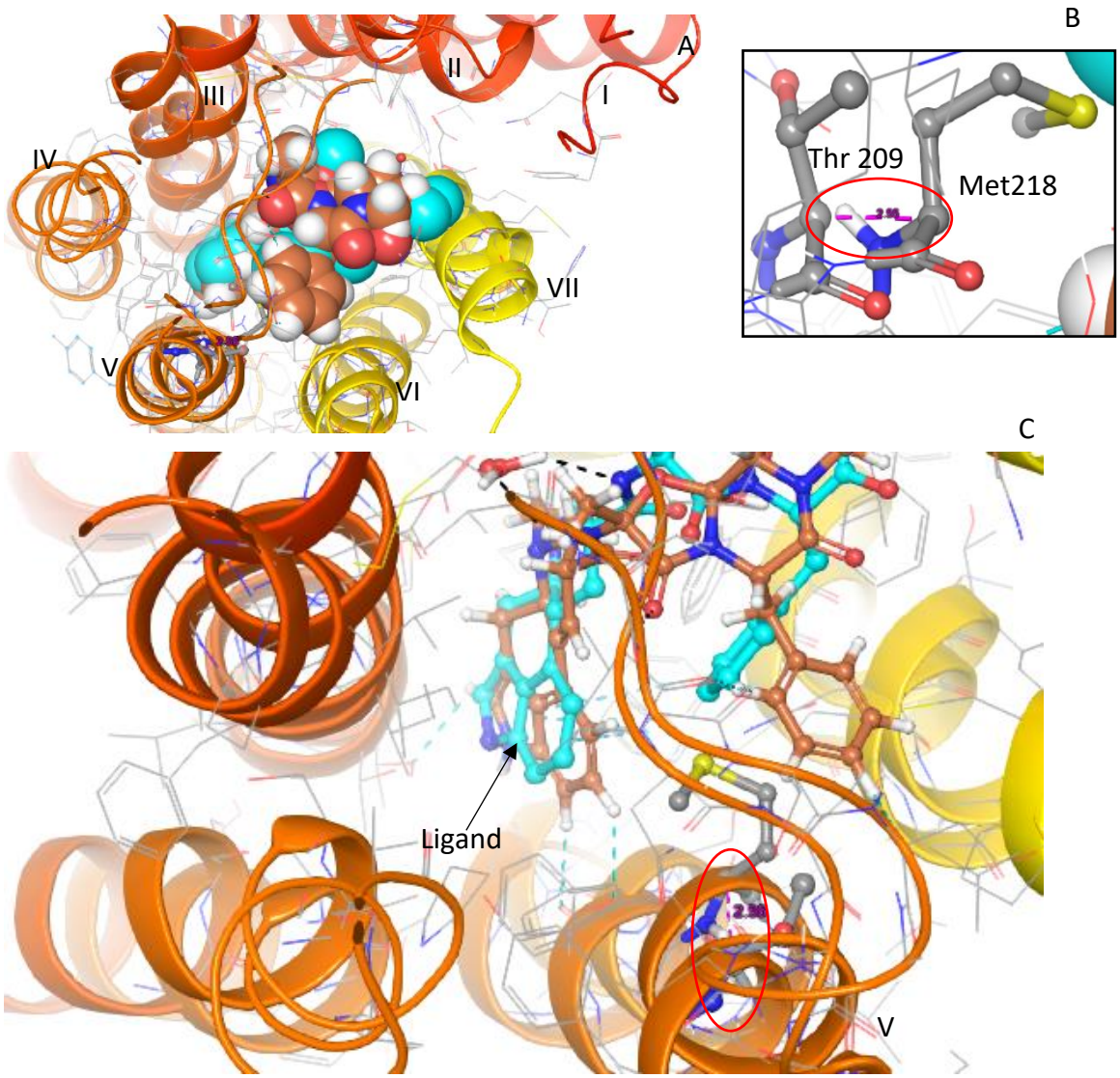

Figure 3.6 A) Topographical view of the extracellular helices of the aligned 5-HTRs with their respective ligands in CPK representation. B) Distance measured between the aligned residues, Thr 209 of the 5-HT1_B and Met 218 of the 5-HT2_B, shows 2.55 Å shift between the α-carbons from helix V. C) Close up view of the LBC with the distance measurement highlighted in red circle.

- A pi-pi stacking interaction observed between the indole ring of the natural ligand and the residue Phe 331, as well as a hydrogen bond along with a salt bridge interaction from the aliphatic amino group side chain to Asp 129 and a pi-cation interaction with Phe 330 was observed during docking experiments in the hydrophobic binding cleft of the 5-HT1_B (Fig. 3.7).

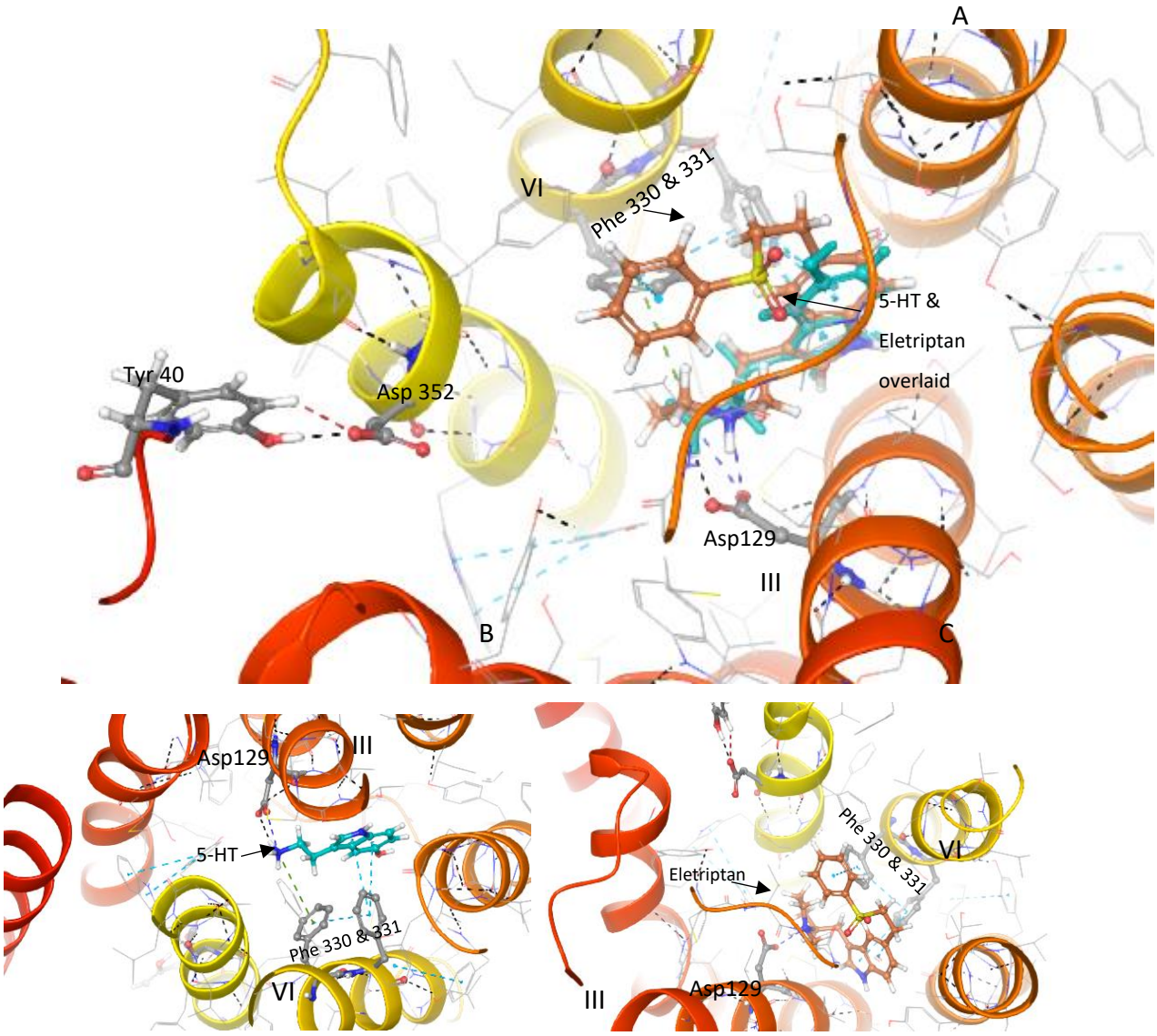

Figure 3.7 Intra receptor and ligand interactions after docking 5-HT (in teal colour ligand) and eletriptan (in orange red ligand) with 4IAQ receptor. All key residues in grey ball & stick representations, black dotted lines represent H-bonding, teal dotted lines represent pi-pi interactions. A) Both ligands in overlaid view to depict similar interactions, blue dotted line from Asp 129 indicates a salt bridge interaction, green dotted line for pi-cationic interaction. B) 5-HT interactions at the LBC C) Eletriptan interactions at the LBC.

3.3.2 Receptor features

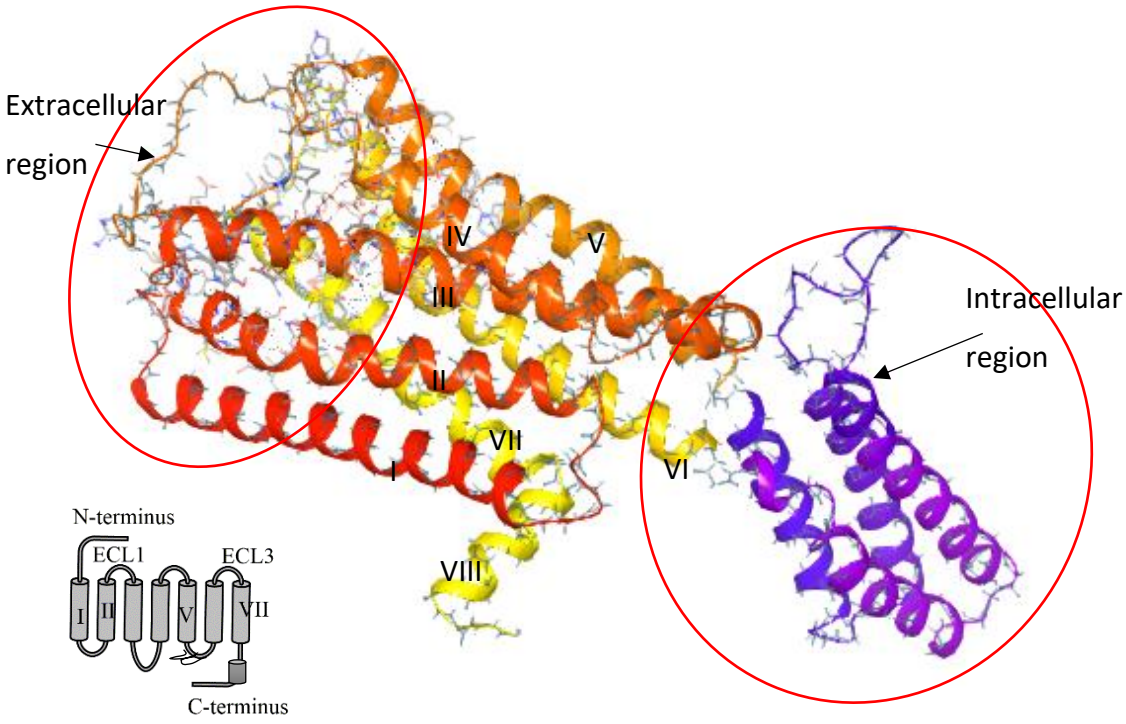

Figure 3.8 5-HT1$_B$ highlighting its extracellular region and the intracellular regions in 3D view. The middle non-highlighted area is assumed to be embedded in the cell membrane region, hence known as transmembrane region. A cartoon image in the inset shows how the helices would look in a planar 2D view.

All 5-HTRs have 7 transmembrane regions known as helices formed by several AA residues. The N-terminus loop from extracellular region continues as helix I and forms an intracellular loop which continues further upward as helix II. Thus 7 helices are formed spanning between extracellular region and intracellular regions comprising of 3 extra cellular loops and 3 intracellular loops excluding the N-terminus loop and C-terminus loop which is assumed to be in the intracellular region. Upon examining the x-ray crystallographic images of 5-HT1$_B$ models using Maestro, helix V continues from the transmembrane region to intracellular region then folding further to form 4 additional transverse membranes within intracellular region before continuing further upward to transmembrane as helix VI. Hence the major part of the intracellular region of the receptor is formed by residues from helix V. The intracellular region then further couples with the G proteins from the cytoplasmic region. All 5-HTRs are known to form a disulphide bridge between residues from ECL2 and helix III. Some 5-HTRs like 5-HT1$_B$ are inhibitory in nature, whereby they control the propagation of signals between synapses among neurons (54).

The differences in an active and inactive conformation state of the receptor can aid in better understanding the interactions and the subsequent transducer recruitments.

3.3.3 Understanding active and inactive conformations and the key residues activating the trigger motifs or microswitches in 5-HTRs

When we study other resolved active and inactive GPCR conformations such as β_2 adrenergic receptors (β_2AR), have demonstrated in the past that certain key residues near the binding pocket, upon agonist induced activation, act as trigger motifs or microswitches, whereby facilitates large-scale helical rearrangements. Structures of 5-$HT1_B$ & 5-$HT2_B$ have been studied in the past in an active confirmation with ERM ligand. However recently in the year 2018 an inactive conformation is known of 5-HT_2C in complex with an inverse agonist, which reveals some understanding of the biased signalling noticed in the past with 5-$HT2_B$ and the key structural differences that facilitate this phenomenon. Many drugs currently researched are actively targeting GPCRs for their involvement in many disorders in the past. 5-HTRs are a new addition in the research interests for the treatment of various disorders including migraine and resolving the structural details may facilitate drug design at multiple GPCRs. A few examples of drugs targeting GPCRs are; opioid analgesics targeting μ opioid receptors, anti-histamines targeting histamine H1 receptors, anti-psychotics targeting DA receptor D_2.

Since this docking experiment used a rigid receptor conformation, to study the conformational changes in 5-HT receptor we need to use more dynamic molecular modelling system. In the absence of molecular dynamic software tool, superimposing the active and inactive conformations of receptors are a great way to study the helical relocations upon activation.

Following residue motifs are important in differentiating an active conformation;

- PIF motif: Pro, Ile and Phe residues from helices V, III, and VI in β_2 adrenergic receptors have been shown to rearrange upon activation (58, 78, 83).
- NPxxY motif: Asn, Pro and Tyr residues involving helix VII of GPCRs (58, 78, 83).
- DRY motif: Asp, Arg and Tyr residues involving helix III (58, 78, 83, 84).

The superimposed PIF motifs from $5HT1_B$ and $5\text{-}HT2_B$ when compared to active β_2AR showed fully active conformations for $5HT1_B$, however $5\text{-}HT2_B$ was indicative of a partially acive conformation (57, 59, 78, 82, 85). This difference was thought to be involved in its functional selectivity or biased signalling property. This was supported by luminescence based assays measuring specific G protein activation (G_i activation & cAMP production) and fluorescence based calcium mobilization assays (to measure G_q activation) compared with β-AR recruitment measuring assays (β-AR dependent luciferase reporter assays in HEK293 cell lines) (78). Several mutational studies along with binding kinetics experiments conducted in previous researchers have identified the important residues (residues from ECL2) involved in $5\text{-}HT2_B$ resulting in longer ligand residence with hallucinogenic drugs such as LSD in contributing to its hallucinogenic properties, hence this understanding has opened new possibilities in structure based drug designs (83).

3.4 Can anti-migraine drugs be more efficient to improve treatment outcome?

In section 1.3.3 lists the total number of known 5-HTRs, and the complexities involved in their signalling mechanisms (section 1.5.4 to 1.5.6). Since the pathogenesis of migraine involves the CNS, and the receptor mechanisms involved in migraine is not fully understood. The signalling mechanism of 5-HTRs involve subtype selectivity, and both inhibitory and agonistic mechanisms involving the diverse receptor subtypes engaging CNS. Structural basis for ligand promiscuity and subtype selectivity involving 5-HTRs is still under research. 2 distinct receptor conformation specific signalling of 5-HTRs have revealed several possible signalling mechanisms involving 5-HTRs. The involvement of DA and NE in migraine and the influence of 5-HT in the regulation of the monoaminergic system needs to be fully elucidated.

Insilico modelling has helped in understanding the key interactions of ligand receptor conformations. Using an active conformation to distinguish the key features in ligand-receptor recognition process, by comparing a mutated or an inactive receptor conformation, has opened more possibilities in structure-based approach using *insilico* modelling studies. Section 2.3 lists superimposed receptor conformations of two different 5-HTR subtypes (refer Fig. 2.9 & 2.10), in two distinct active conformations. The extended

binding pockets were crucial in distinguishing these two receptors, where the binding pockets were narrower compared to the other, forcing the ligand to occupy unfavourable positions in one of the receptors.

3.5 Conclusions

An understanding of the receptor characteristics and activation mechanisms may ultimately facilitate better drug designs in the future; however, the pathogenesis of migraine needs to be fully elucidated to facilitate better drug targeting in the treatment of this disorder. A successful drug design should incorporate the diverse pharmacological aspects involving agonistic, antagonistic or inverse agonistic activity at the target receptor. From the studies discussed in section 1.3.6, it is possible to link DA/5-HT levels in the brain to migraine development. 5-HT/DA have affinities to a wide range of receptor subtypes, the same could also be true for anti-migraine drugs which may act as neurotransmitters or DA/5-HT mimics (due to structural analogy) and possibly be correcting the systemic neurotransmitter imbalance by binding to the unoccupied receptors to activate them. Therefore, anti-migraine drugs could act as 5-HT or DA mimics. This research thesis has indicated this structural analogy clearly via the *insilico* receptor interaction simulations. To test whether these *insilico* experiments can be reproduced in real systems, in vivo experiments (using tissue culture or animal experiments) need to be supported to confirm the findings. This research could potentially fill the gaps in our understanding of the underlying pathogenesis of migraine and the interactions of anti-migraine drugs with key migraine associated neuroreceptors. Thus, this research might help to develop better targeted treatment alternatives for migraine, in the future.

(2, 9, 10, 13, 14, 16, 19, 26, 28, 33, 35, 36, 38-40, 42-44, 46, 48-50, 54, 55, 60, 63-66, 72, 79, 80, 85-111).

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